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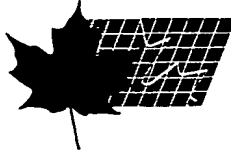
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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Phosphonomonoester Nucleic Acids, Process for Their Preparation, and Their Use

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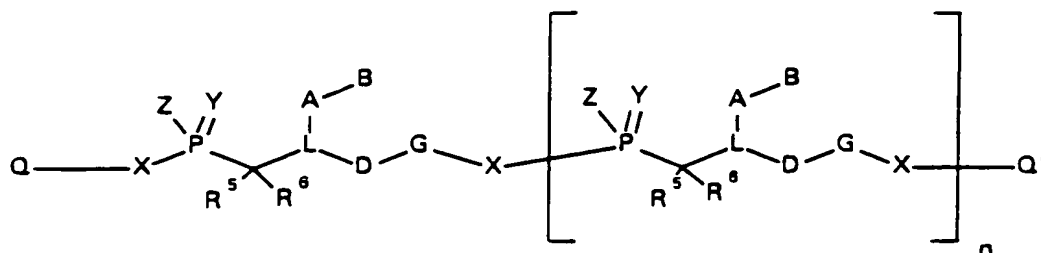
(57) 49 Claims

Notice: This application is as filed and may therefore contain an incomplete specification.



ABSTRACT OF THE DISCLOSURE

Novel oligonucleotide analogs of the formula (I) are described,



(I)

in which A, B, D, G, L, P, Q, Q', R⁵, R⁶, X, Y, Z and n are as defined in the description, which have useful physical, biological and pharmacological properties, as well as a process for their preparation. Their application relates to use as inhibitors of gene expression (antisense oligonucleotides, ribozymes, sense oligonucleotides and triplex-forming oligonucleotides), as probes for the detection of nucleic acids and as auxiliaries in molecular biology.

PHOSPHONOMOESTER NUCLEIC ACIDS,
PROCESS F R THEIR PREPARATI N, AND THEIR USE

5 The present invention relates to novel oligonucleotide
analogs having useful physical, biological and pharmaco-
logical properties, and to a process for their preparation.
Their application relates to use as inhibitors of gene
10 expression (antisense oligonucleotides, ribozymes, sense
oligonucleotides and triplex-forming oligonucleotides), as
probes for the detection of nucleic acids and as auxiliaries
in molecular biology.

15 The disclosure of all documents referred to in this
specification are incorporated in whole by reference.

Oligonucleotides are applied to a growing extent as
inhibitors of gene expression (J. F. Milligan,
20 M. D. Matteucci and J. C. Martin, J. Med. Chem. 36 (1993)
1923; E. Uhlmann and A. Peyman, Chemical Reviews 90 (1990)
543; S. T. Crooke, Annu. Rev. Pharmacol. Toxicol. 32 (1992)
329).

25 Antisense oligonucleotides are nucleic acid fragments whose
base sequence is complementary to an mRNA to be inhibited.
This target mRNA can be of cellular, viral or other
pathogenic origin. Possible cellular target sequences are,
for example, those of receptors, enzymes, growth factors,
immunomodulators, ion channels or oncogenes. The inhibition
30 of virus replication with the aid of antisense
oligonucleotides was described, for example, for RSV (rous
sarcoma virus), HSV-1 and -2 (herpes simplex virus type I
and II), HIV (human immunodeficiency virus) and influenza
viruses. In this case, oligonucleotides are employed which
35 are complementary to the viral nucleic acid.

The sequence of sense oligonucleotides is designed, however, such that they bind ("capture"), for example, nucleic acid-binding proteins or nucleic acid-processing enzymes and thus inhibit their biological activity (C. Hélène and J. J. Toulmé, *Biochim. Biophys. Acta* 1049 (1990) 99). Viral targets which may be mentioned here are, for example, reverse transcriptase, DNA polymerase and transactivator proteins. In general, triplex-forming oligonucleotides have DNA as a target and, after binding to this, form a triple helical structure.

Whereas with the aid of the antisense oligonucleotides, the processing (splicing etc.) of the mRNA or its translation into the protein are in general inhibited, triplex-forming oligonucleotides inhibit the transcription or replication of the DNA (N. T. Thuong, and C. Hélène, *Angew. Chem.* 105 (1993) 697; Uhlmann and Peyman, *Chemical Reviews* 90 (1990) 543). However, it is also possible to bind single-stranded nucleic acids with an antisense oligonucleotide in a first hybridization with formation of a double strand, which then forms a triplex structure in a second hybridization with a triplex-forming oligonucleotide. The antisense and triplex-binding regions can in this case either be accommodated in two separate oligonucleotides or else in one oligonucleotide.

A further application of synthetic oligonucleotides are the so-called ribozymes, which destroy the target RNA as a result of their ribonuclease activity (D. Castanotto, J. J. Rossi, J. O. Deshler, *Critical Rev. Eukar. Gene Expr.* 2 (1992) 331).

In DNA diagnosis, nucleic acid fragments with suitable labelling are employed as so-called DNA probes for the specific hybridization of a nucleic acid to be detected. The specific formation of the new double strand is in this case

monitored with the aid of the labelling, which is preferably not radioactive. In this manner, genetic, malignant, viral or other pathogen-caused diseases can be detected.

5 For most applications mentioned, oligonucleotides in their naturally occurring form are not very suitable or completely unsuitable. They must be chemically modified such that they meet the specific requirements. In order that
10 oligonucleotides can be employed in biological systems, for example for the inhibition of virus replication, they must fulfill the following requirements:

1. They must have a sufficiently high stability under in vivo conditions, i.e. both in serum and intracellularly.

15 2. They must be constituted such that they can pass through the cell and nucleus membrane.

3. They must bind to their target nucleic acid in base-specific manner under physiological conditions in order to display the inhibitory effect.

20 These requirements are not indispensable for DNA probes; however, these oligonucleotides must be derivatized such that detection is possible, for example by means of fluorescence, chemiluminescence, colorimetry or specific
25 staining (Beck and Köster, Anal. Chem. 62 (1990) 2258).

A multiplicity of chemical variations of oligonucleotides are known which have been synthesized with the aim of fulfilling the abovementioned requirements better than the
30 unmodified oligonucleotides. The chemical modification of the oligonucleotides is usually carried out by appropriately modifying the phosphate backbone, ribose unit or the nucleobases (Uhlmann and Peyman, Chemical Review 90 (1990) 543). The modifications also include those in which both the
35 phosphate bridge and the sugar unit have been replaced by other groups, for example by "morpholinonucleoside"

oligomers (E. P. Stirchak et al., Nucleic Acids Res. 17 (1989) 6129) or "PNAs" (P. E. Nielsen et al, Bioconj. Chem. 5 (1994) 3). PNAs, in particular, are distinguished by unusually high affinities for target RNA, but suffer from other unfavorable properties such as lack of solubility or deficient cell penetration (W. Wang et al., Tetrahedron Letters 36 (1995) 1181; M. Egholm et al., in "Innovation and Perspectives in Solid Phase Synthesis, Peptides, Proteins, Nucleic Acids", Roger Epton, Ed. Mayflower Worldwide Limited, Birmingham, 1994, 145-148).

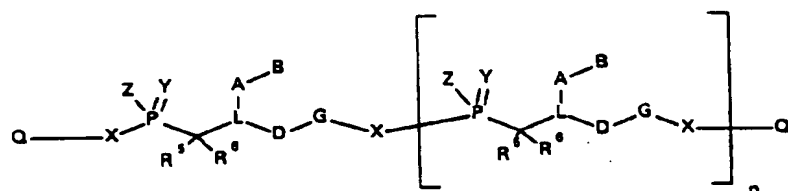
It is therefore an object to find novel oligonucleotide analogs having favorable properties.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a graph showing a UV-Absorbance Profile Versus Temperature for [pmena-t9] in the Presence of 1 eq. dA9 [See Example 56].

Figure 2 is a chart showing proof of PMENA-DNA binding by Gel-Shift [See Example 57].

The invention therefore relates to compounds of the formula I



(I)

wherein n is a number from zero to 100;

B independently of one another is hydrogen, hydroxyl, (C₁-C₂₀)-alkyl, (C₁-C₂₀)-alkoxy, (C₁-C₂₀)-alkylthio, (C₆-C₂₀)-aryl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkyl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkoxy, (C₆-C₂₀)-aryl-(C₁-C₆)-alkylthio, an aromatic group or a heterocyclic group, where alkyl, aryl, the aromatic or heterocyclic group can optionally be substituted one or more times by hydroxyl, (C₁-C₄)-alkoxy, -NR⁹R¹⁰, -C(O)OH, oxo, -C(O)OR⁸, -C(O)NR⁹R¹⁰, -CN, -F, -Cl, -Br, -NO₂, (C₂-C₆)-alkoxyalkyl, -S(O)_mR⁸, - (C₁-C₆)-alkyl- S(O)_mR⁸, -NHC(=NH)NHR⁸, -C(=NH)NHR⁸, -NR⁹C(=O)R⁸, =NOR⁸, NR⁹C(=O)OR¹⁰, -OC(=O)NR⁹R¹⁰ or -NR⁹C(=O)NR⁹R¹⁰,

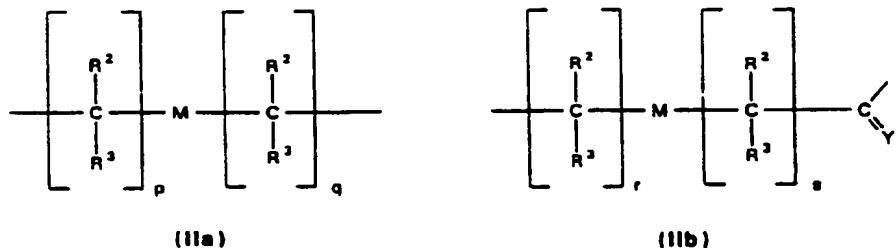
B independently of one another is a natural nucleobase, an unnatural nucleobase or a reporter ligand; and

A-B can also be a D- or L-amino acid condensed on via the carboxyl group or peptides consisting of these amino acids having a length of up to 5 amino acid residues,

L independently of one another is N or R¹N^{*}, and

R¹ is hydrogen or (C₁-C₆)-alkyl which can be substituted by hydroxyl, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio or amino. Preferably, R¹ is hydrogen or methyl;

A independently of one another is a single bond, a methylene group or a group of the formula IIa or IIb;



Y' is =O, =S, =CH₂, =C(CH₃)₂ or =NR¹, where R¹ is as defined above;

M is a single bond, -O-, -S- or -NR¹-, where R¹ is as defined above;

R² and R³ independently of one another are hydrogen, hydroxyl, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio, amino, halogen, such as F, Cl or Br, or (C₁-C₆)-alkyl which can optionally be substituted by hydroxyl, (C₁-C₆)-alkoxy or (C₁-C₆)-alkylthio, but are preferably hydrogen;

p and q independently of one another are zero to 5;

r and s independently of one another are zero to 5;

D and G each represent CR⁵R⁶ which can be the same or different;

R⁵ and R⁶ independently of one another are hydrogen, (C₁-C₆)-alkyl, (C₆-C₂₀)-aryl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkyl, hydroxyl, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio, and alkyl and aryl can optionally be substituted by SR¹ or NR¹R^{1'}, where R¹ is as defined above and R^{1'} independently of R¹ has the same meaning as R¹, but R⁵ and R⁶ are preferably hydrogen;

X is -O-, -S- or -NR¹-, in which R¹ is as defined above;

Y is =O or =S;

Z is -OR⁸, -NR⁹R¹⁰ or X'Q", where X' is defined as X and Q" is defined as Q;

R⁸ is hydrogen, (C₁-C₁₈)-alkyl, (C₂-C₁₈)-alkenyl, (C₃-C₁₈)-alkynyl, (C₆-C₁₂)-aryl, (C₆-C₁₂)-aryl-(C₁-C₆)-alkyl, where alkyl can be substituted one or more times by hydroxyl, (C₁-C₄)-alkoxy, F, Cl or Br and aryl can be substituted 1-3 times by hydroxyl, (C₁-C₄)-alkoxy, (C₁-C₄)-alkyl, F, Cl, Br, NO₂, -NR⁹R¹⁰, -C(O)OH, -C(O)O-(C₁-C₆)-alkyl or -C(O)NR⁹R¹⁰, but preferably R⁸ is hydrogen, (C₁-C₆)-alkyl, (C₆-C₁₂)-aryl or (C₆-C₁₂)-aryl-(C₁-C₆)-alkyl, where aryl can be monosubstituted by (C₁-C₄)-alkoxy, (C₁-C₄)-alkyl, F, Cl, Br or NO₂. More preferably R⁸ is hydrogen, (C₁-C₆)-alkyl, phenyl or 2-(4-nitrophenyl)ethyl;

R⁹ and R¹⁰ independently of one another are hydrogen, (C₁-C₁₈)-alkyl, (C₁-C₁₈)-alkenyl, (C₁-C₁₈)-alkynyl, (C₆-C₁₂)-aryl, (C₆-C₁₂)-aryl-(C₁-C₆)-alkyl, where alkyl can be substituted one or more times by hydroxyl, (C₁-C₄)-alkoxy, F, Cl or Br, or R⁹ and R¹⁰ can together form a 4 to 7-membered ring together with the N atom carrying them;

Q and Q' independently of one another are hydrogen or R⁸.

Alternatively, Q and Q' are conjugates which a) favorably affect the properties of antisense oligonucleotides, b) affect the properties of triple helix-forming oligonucleotides, c) serve as a label of a DNA probe, d) during the hybridization of the oligonucleotide analog to the target nucleic acid, attack the target nucleic acid through binding or crosslinking. Or, Q and Q' are oligonucleotides which can be unmodified or modified, where the following variants are intended to be examples of some modifications (e.g. described in E. Uhlmann and A. Peyman, Chemical Reviews 90 (1990) 543; "Protocols for Oligonucleotides and Analogs", Synthesis and Properties &

Synthesis and Analytical Techniques, S. Agrawal, Ed, Humana Press, Totowa, USA 1993):

a) complete or partial replacement of the 3'- and/or the 5'-phosphoric acid diester bridges, for example by phosphorothioate, phosphorodithioate, $NR^4R^{4'}$ -phosphoramidate, boranophosphate, phosphate- (C_1-C_{21}) -O-alkyl ester, phosphate- $[(C_6-C_{12})$ aryl- (C_1-C_{21}) -O-alkyl] ester, 2,2,2-trichlorodimethylethylphosphonate, (C_1-C_8) alkylphosphonate or (C_6-C_{12}) -arylphosphonate bridges, where R^4 and $R^{4'}$ independently of one another are hydrogen, (C_1-C_{18}) -alkyl, (C_6-C_{20}) -aryl, (C_5-C_{14}) -aryl- (C_1-C_8) -alkyl or $-(CH_2)_c-[NH(CH_2)_d]_d-NR^7R^7$, in which c is an integer from 2 to 6 and d is an integer from 0 to 6, and R^7 independently of one another is hydrogen, (C_1-C_6) -alkyl or (C_1-C_4) -alkoxy- (C_1-C_6) -alkyl, preferably R^4 and $R^{4'}$ are hydrogen, (C_1-C_8) -alkyl or methoxyethyl, particularly preferably hydrogen, (C_1-C_4) -alkyl or methoxyethyl or R^4 and $R^{4'}$, together with the nitrogen atom carrying them, can also form a 5 to 6-membered heterocyclic ring which can additionally contain a further heteroatom from the series consisting of O, S and N;

b) complete or partial replacement of the 3'- or 5'-phosphoric acid diester bridges by "dephospho" bridges (see, for example, Uhlmann and Peyman in "Methods in Molecular Biology", Vol. 20, "Protocols for Oligonucleotides and Analogs", S. Agrawal, Ed., Humana Press, Totowa 1993, Chapter 16, 355ff), for example by formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylene-dimethylhydrazo, dimethylenesulfone or silyl groups;

c) complete or partial replacement of the sugar phosphate backbone, for example by "morpholinonucleoside" oligomers (E. P. Stirchak et al., Nucleic Acids Res. 17 (1989) 6129) or "PNAs" (P. E. Nielsen et al, Bioconj. Chem. 5 (1994) 3), or PNA-DNA hybrids such as described, for

example, in DE-P 44 08 528.1 and EP-A 0 672 677 (HOE 94/F 057);

d) complete or partial replacement of the β -D-2'-deoxyribose units, for example by α -D-2'-deoxyribose, L-2'-deoxyribose, 2'-F-2'-deoxyribose, 2'-O-(C₁-C₆)alkyl-ribose, 2'-O-(C₂-C₆)alkenylribose, 2'-NH₂-2'-deoxyribose, β -D-xylofuranose, α -arabinofuranose, 2,4-dideoxy- β -D-erythrohexopyranose, and carbocyclic (e.g. Froehler, J. Am. Chem. Soc. 114 (1992) 8320) and open-chain sugar analogs (e.g. Vandendriessche et al., Tetrahedron 49 (1993) 7223) or bicyclo sugar analogs (e.g. M. Tarkov et al., Helv. Chim. Acta 76 (1993) 481);

e) complete or partial replacement of the natural nucleoside bases, for example by 5-(hydroxymethyl)uracil, 5-aminouracil, pseudouracil, dihydrouracil, 5-(C₁-C₆)-alkyluracil, 5-(C₂-C₆)-alkenyluracil, 5-(C₂-C₆)-alkynyluracil (for example described in Gutierrez et al., J. Am. Chem. Soc. 116 (1994) 540 or Sagi et al., Tetrahedron Lett. 34 (1993) 2191), 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-(C₂-C₆)-alkynylcytosine (Gutierrez et al., J. Am. Chem. Soc. 116 (1994) 540 or Sagi et al., Tetrahedron Lett. 34 (1993) 2191), 5-fluorouracil, 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine or 7-deaza-7-substituted purines (for example described in Seela, Nucl. Acids Res. 20 (1992) 2297); Heterocycles 34 (1992) 229).

Q and Q' can also be conjugates which favorably affect the properties of antisense oligonucleotides or of triple helix-forming oligonucleotides (such as, for example, cell penetration, nuclease degradation, affinity for the target RNA/DNA, pharmacokinetics) or serve as a label of a DNA probe or during the hybridization of the oligonucleotide analog to the target nucleic acid, attacking the target

nucleic acid through binding or crosslinking. Examples of these are conjugates with polylysine, with intercalators such as pyrene, acridine, phenazine, phenanthridine, with fluorescent compounds such as fluorescein, with crosslinkers such as psoralen, azidoproflavine, with lipophilic molecules such as (C₁₂-C₂₀)-alkyl, with lipids such as 1,2-dihexadecyl-rac-glycerol, with steroids such as cholesterol or testosterone, with vitamins such as vitamin E, with poly- or oligoethylene glycol, with (C₁₂-C₁₈)-alkyl phosphate diesters, with -O-CH₂-CH(OH)-O-(C₁₂-C₁₈)-alkyl. Preferred conjugates are those with lipophilic molecules such as (C₁₂-C₂₀)-alkyl, with steroids such as cholesterol or testosterone, with poly- or oligoethylene glycol, with vitamin E, with intercalators such as pyrene, with (C₁₄-C₁₈)-alkylphosphate diesters or with -O-CH₂-CH(OH)-O-(C₁₂-C₁₈)-alkyl.

The preparation of oligonucleotide conjugates of this type is known to the person skilled in the art (see, for example, Uhlmann & Peyman, Chem. Rev. 90 (1990) 543; M. Manoharan in "Antisense Research and Applications", Crooke and Lebleu, Eds., CRC Press, Boca Raton, 1993, Chapter 17, p.303ff and EP-A 0 552 766). Furthermore, the oligonucleotides can carry 3'-3'- and 5'-5'-inversions on the 3' or on the 5'-end (described, for example, in M. Koga et al., J. Org. Chem. 56 (1991) 3757).

Aromatic groups are, for example, phenyl, naphthyl, pyrenyl, anthracenyl, phenanthryl, biphenyl, binaphthyl, tetracenyl, pentacenyl, hexacenyl, triphenylenyl, chrysenyl or benzopyrenyl.

Heterocyclic groups are understood as meaning, for example, chromanyl, chromenylium-1-yl, furanyl, isochromanyl, isochromenyl, isoquinolyl, piperazinyl, quinolinyl, pyridinyl, pyrrolidinyl, imidazolyl, tetrahydrofuranyl, aziridinyl, oxiranyl, thiophenyl, pyrimidinyl, thiolanyl,

thiazolyl, azepinyl, pyrrolyl, tetrahydropyrrolyl,
 benzofuranyl, indolyl, isoindolyl, isatiny, dioxindolyl,
 indoxyl, coumarinyl, coumaronyl, carbazolyl, pyrazolyl,
 pyrrolyl, indazolyl, oxazolyl, isoxazolyl, thiazolyl, 1,2,4-
 triazolyl, 1,2,3-triazolyl, tetrazolyl, pentazolyl,
 piperidinyl, pyridazinyl, phenazinyl, phenoxazinyl,
 phenothiazinyl, morpholinyl, thiazinyl, benzodiazepinyl,
 purinyl, xanthinyl, hypoxanthinyl, theophyllinyl,
 theobrominyl, caffeinyl, pteridinyl, pterinyl, pteridinyl,
 alloxazinyl and nortropinyl.

Natural nucleobases are understood as meaning, for example,
 uracil, cytosine, 5-methyluracil, adenine and guanine and
 unnatural nucleobases are understood as meaning, for
 example, 5-nitroindole, 5-(hydroxymethyl)-uracil, 5-
 aminouracil, pseudouracil, dihydrouracil, 5-(C₁-C₆)-
 alkyluracil, 5-(C₂-C₆)-alkenyluracil, 5-(C₃-C₆)-
 alkynyluracil, 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenyl-
 cytosine, 5-(C₃-C₆)-alkynylcytosine, 5-fluorouracil,
 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine,
 5-bromouracil, 5-bromocytosine and 7-deaza-7-substituted
 purines, such as 7-deaza-7-(C₃-C₇)-alkynylguanine, 7-deaza-
 7-(C₃-C₇)-alkynyladenine, 7-deaza-7-(C₂-C₇)-alkenylguanine,
 7-deaza-7-(C₂-C₇)-alkenyladenine, 7-deaza-7-(C₁-C₇)-
 alkylguanine, 7-deaza-7-(C₁-C₇)-alkyladenine, 7-deaza-7-
 bromoguanine and 7-deaza-7-bromoadenine.

Preferably, unnatural nucleobases are 5-(C₁-C₆)-alkyluracil,
 5-(C₂-C₆)-alkenyluracil, 5-(C₃-C₆)-alkynyluracil, 5-
 (C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-(C₃-C₆)-
 alkynylcytosine, 5-fluorouracil, 5-fluorocytosine, 5-
 chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromo-
 cytosine or 7-deaza-7-substituted purines such as 7-deaza-7-
 (C₃-C₇)-alkynylguanine, 7-deaza-7-(C₃-C₇)-alkynyladenine, 7-
 deaza-7-(C₂-C₇)-alkenylguanine, 7-deaza-7-(C₂-C₇)-
 alkenyladenine, 7-deaza-7-(C₁-C₇)-alkylguanine, 7-deaza-7-

(C₁-C₇)-alkyladenine, 7-deaza-7-bromoguanine and 7-deaza-7-bromoadenine, particularly preferably 5-(C₃-C₆)-alkyluracil, 5-(C₂-C₆)-alkenyl-uracil, 5-(C₃-C₆)-alkynyluracil, 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-(C₃-C₆)-alkynylcytosine or 7-deaza-7-substituted purines, and very particularly preferably 5-pentynylcytosine, 5-hexynyluracil, 5-hexynylcytosine, 7-deaza-7-propynylguanine, 7-deaza-7-propynyladenine, 7-deaza-7-methylguanine, 7-deaza-7-methyladenine, 7-deaza-7-propynyladenine, 7-deaza-7-bromoguanine, 7-deaza-7-bromoadenine.

Reporter ligands are, for example, fluorescein, biotin, acridine, phenanthroline, phenanthridine and eosin.

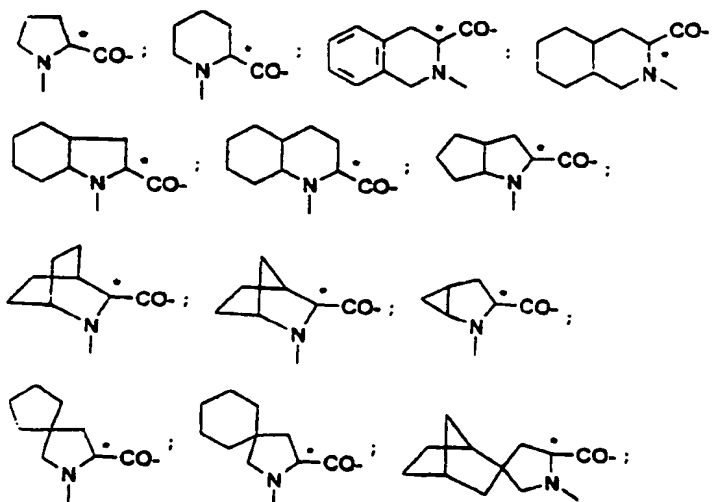
Among D- or L-amino acids, if not stated otherwise, particularly the following may be mentioned (cf. Schröder, Lübke, Peptides, Volume 1, New York 1965, pages XXII-XXIII; Houben-Weyl, Methoden der Organischen Chemie, [Methods of Organic Chemistry] Volume XV/1 and 2, Stuttgart 1974):

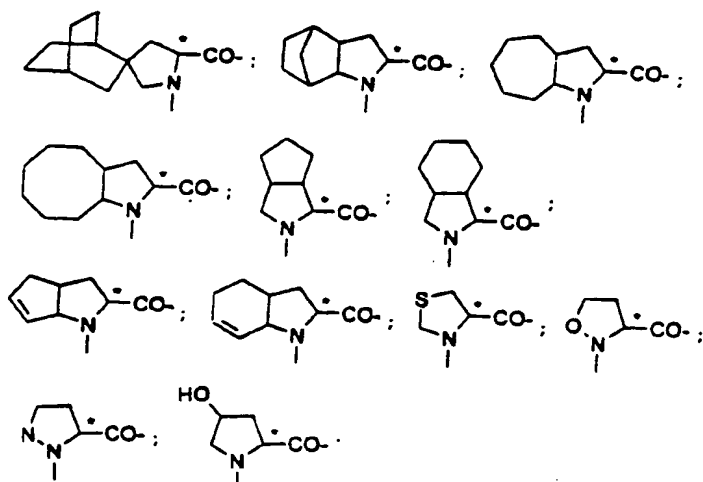
Aad, Abu, γAbu, ABz, 2ABz, εAca, Ach, Acp, Adpd, Ahb, Aib, βAib, Ala, βAla, ΔAla, Alg, All, Ama, Amt, Ape, Apm, Apr, Arg, Asn, Asp, Asu, Aze, Azi, Bai, Bph, Can, Cit, Cys, Cyta, Daad, Dab, Dadd, Dap, Dapm, Dasu, Djen, Dpa, Dtc, Fel, Gln, Glu, Gly, Guv, hAla, hArg, hCys, hGln, hGlu, His, hIle, hLeu, hLys, hMet, hPhe, hPro, hSer, hThr, hTrp, hTyr, Hyl, Hyp, 3Hyp, Ile, Ise, Iva, Kyn, Lant, Lcn, Leu, Lsg, Lys, βLys, ΔLys, Met, Mim, Min, hArg, Nle, Nva, Oly, Orn, Pan, Pec, Pen, Phe, Phg, Pic, Pro, ΔPro, Pse, Pya, Pyr, Pza, Qin, Ros, Sar, Sec, Sem, Ser, Thi, βThi, Thr, Thy, Thx, Tia, Tle, Tly, Trp, Trta, Tyr, Val etc., whose abbreviations without a stereo-descriptor stand for the radical in the L-form,

and cyclic amino acids, such as

pyrrolidine-2-carboxylic acid; piperidine-2-carboxylic acid; 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid;

- decahydroisoquinoline-3-carboxylic acid; octahydroindole-2-carboxylic acid; decahydroquinoline-2-carboxylic acid; octahydrocyclopenta[b]pyrrole-2-carboxylic acid; 2-azabicyclo[2.2.2]octane-3-carboxylic acid;
- 5 2-azabicyclo[2.2.1]heptane-3-carboxylic acid; 2-azabicyclo[3.1.0]-hexane-3-carboxylic acid; 2-azaspiro[4.4]nonane-3-carboxylic acid; 2-azaspiro[4.5]decane-3-carboxylic acid;
- 10 spiro[(bicyclo[2.2.1]heptane)-2,3-pyrrolidine-5-carboxylic acid]; spiro[(bicyclo[2.2.2]octane)-2,3-pyrrolidine-5-carboxylic acid]; 2-azatricyclo[4.3.0.1^{6,9}]decane-3-carboxylic acid; decahydrocyclohepta[b]pyrrole-2-carboxylic acid;
- 15 decahydrocycloocta[b]pyrrole-2-carboxylic acid; octahydrocyclopenta[c]pyrrole-2-carboxylic acid; octahydroisindole-1-carboxylic acid; 2,3,3a,4,6a-hexahydrocyclopenta[b]pyrrole-2-carboxylic acid; 2,3,3a,4,5,7a-hexahydroindole-2-carboxylic acid;
- 20 tetrahydrothiazole-4-carboxylic acid; isoxazolidine-3-carboxylic acid; pyrazolidine-3-carboxylic acid; hydroxypyrrolidine-2-carboxylic acid; all of which can be optionally substituted with:





US Patent Nos. 4,344,949, 4,374,847, and 4,350,704, and
 EP-A 29 488, EP-A 31 741, EP-A 46 953, EP-A 49 605, EP-A 49
 658, EP-A 50 800, EP-A 51 020, EP-A 52 870, EP-A 79 022,
 EP-A 84 164, EP-A 89 637, EP-A 90 341, EP-A 90 362, EP-A 105
 102, EP-A 109 020, EP-A 111 873, EP-A 271 865 and EP-A 344
 682 describe cyclic amino acids.

In formula I even when n is greater than 1, the
 substitutions can be the same or different from each other.

Alkyl and radicals derived therefrom such as alkoxy and
 alkylthio can be branched, unbranched or cyclic, saturated
 or mono- or polyunsaturated.

Preferred compounds of the formula I are those wherein

n is a number from zero to 50;

B independently of one another is a natural nucleobase or
 an unnatural nucleobase;

L is N;

A is a group of the formula IIb, in which
 r = 1 and s is zero, and R², R³ = H and Y' = O and M is
 a single bond;

D and G each independently represent CHR^5 ;

R^5 is hydrogen;

5 X is -O-;

Y is =O;

10 Z is hydroxyl, methoxy, ethoxy, (4-nitrophenyl)ethoxy, propoxy, isopropoxy, butoxy, pentoxy, phenoxy or allyloxy;

Q and Q' independently of one another are hydrogen, R^8 or oligonucleotides unmodified or modified, as follows:

15

a) the 3'- and/or 5'-phosphoric acid diester bridges are completely or partially replaced by phosphorothioate, phosphorodithioate, $\text{NR}^4\text{R}'$ -phosphoramidate, $\text{N3}'\text{-P5}'$ -phosphoramidate (for example described in Gryaznov et al., J. Am. Chem. Soc. 116 (1994) 3143), phosphate O-methyl ester, phosphate O-ethyl ester, phosphate O-isopropyl ester, methylphosphonate or phenylphosphonate bridges;

20

25 b) one, two or three 3'- or 5'-phosphoric acid diester bridges in the pyrimidine positions and at the 5'-end and/or at the 3'-end are replaced by formacetals and/or 3'-thioformacetals;

30

c) the sugar phosphate backbone is completely or partially replaced by "PNAs" or PNA-DNA hybrids;

35

d) the β -D-2'-deoxyribose units are completely or partially replaced by 2'-F-2'-deoxyribose, 2'-O-($\text{C}_1\text{-C}_6$)-alkylribose, 2'-O-($\text{C}_2\text{-C}_6$)alkenylribose or 2'- NH_2 -2'-deoxyribose;

e) the natural nucleoside bases are completely or partially replaced by 5-(C₁-C₆)-alkyluracil, 5-(C₂-C₆)-alkenyluracil, 5-(C₂-C₆)-alkynyluracil, 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-(C₂-C₆)-alkynylcytosine, 5-fluorouracil, 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine, 7-deaza-7-(C₂-C₇)-alkynylguanine, 7-deaza-7-(C₂-C₇)-alkynyladenine, 7-deaza-7-(C₂-C₇)-alkenylguanine, 7-deaza-7-(C₂-C₇)-alkenyladenine, 7-deaza-7-(C₁-C₇)-alkylguanine, 7-deaza-7-(C₁-C₇)-alkyladenine, 7-deaza-7-bromoguanine, 7-deaza-7-bromoadenine.

Particularly preferred compounds of the formula I are those in which

n is a number from 0 to 30;

Q and Q' independently of one another are hydrogen, R⁸, in which R⁸ is H, (C₁-C₆)-alkyl, phenyl or 2-(4-nitrophenyl)ethyl, or are oligonucleotides which can be unmodified or modified as follows:

a) the 3'- and/or 5'-phosphoric acid diester bridges are completely or partially replaced by phosphorothioate, phosphorodithioate or methylphosphonate bridges;

b) one, two or three 3'- or 5'-phosphoric acid diester bridges are replaced at the 5'- and at the 3'-end;

c) the sugar phosphate backbone is completely or partially replaced by "PNAs" or PNA-DNA hybrids;

d) the β-D-2'-deoxyribose units are completely or partially replaced by 2'-F-2'-deoxyribose, 2'-O-(C₁-C₄)-alkylribose, 2'-O-(C₂-C₄)-alkenylribose or 2'-NH₂-2'-deoxyribose;

e) the natural nucleoside bases are completely or partially replaced by 5-(C₃-C₆)-alkyluracil, 5-(C₂-C₆)-alkenyluracil, 5-(C₂-C₆)-alkynyluracil, 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-(C₂-C₆)-alkynylcytosine, 7-deaza-7-(C₂-C₇)-alkynylguanine, 7-deaza-7-(C₂-C₇)-alkynyladenine, 7-deaza-7-(C₂-C₇)-alkenylguanine, 7-deaza-7-(C₂-C₇)-alkenyladenine, 7-deaza-7-(C₁-C₇)-alkylguanine, 7-deaza-7-(C₁-C₇)-alkyladenine, 7-deaza-7-bromoguanine, 7-deaza-7-bromoadenine.

Very particularly preferred compounds of the formula I are those in which

n is a number from 0 to 25;

B independently of one another is a natural nucleobase;

Z is hydroxyl, ethoxy, (4-nitrophenyl)ethoxy or phenoxy;

Q and Q' independently of one another is hydrogen, R⁸, in which R⁸ is H, (C₁-C₆)-alkyl, phenyl or 2-(4-nitrophenyl)ethyl, or are oligonucleotides which can be unmodified or modified as follows,

a) the 3'- and/or 5'-phosphoric acid diester bridges are completely or partially replaced by phosphorothioate bridges;

c) the sugar phosphate backbone is completely or partially replaced by "PNAs" or PNA-DNA hybrids;

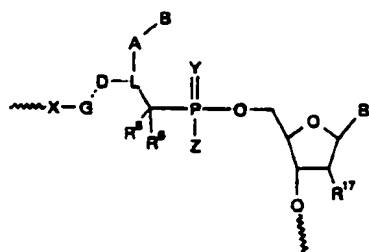
d) the β -D-2'-deoxyribose units are completely or partially replaced by 2'-O-methyl, 2'-O-allyl or 2'-O-butylribose; or

e) the natural nucleoside bases are completely or partially replaced by 5-hexynylcytosine, 5-hexynyluracil, 5-hexynylcytosine, 7-deaza-7-propynylguanine, 7-deaza-7-propynyladenine, 7-deaza-7-methylguanine, 7-deaza-7-methyladenine, 7-deaza-7-bromoguanine, 7-deaza-7-bromo-adenine.

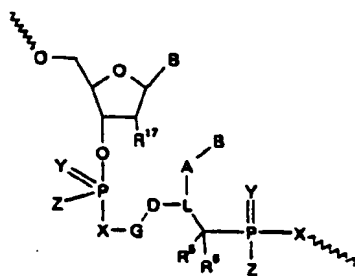
The invention further relates to compounds of the formula I in which Q and Q' are linked, i.e. form a cyclic molecule, where the possibility should still apply that Q and Q' together afford a single bond. The synthesis of such compounds can be carried out analogously to described processes, for example Gao et al., Nucl. Acids Res. 23 (1995) 2025 or Wang and Kool, Nucl. Acids Res. 22 (1994) 2326.

The invention further relates to oligonucleotides or modified oligonucleotides, for example PNAs, in which compounds of the formula I are incorporated at the 3'-end or at the 5'- end or at the 5'- and at the 3'-end.

The linkage of the oligonucleotides with the compounds of the formula I is preferably carried out via the 5'- or 3'-hydroxyl group of the nucleotide units, likewise via a phosphonic acid monoester bond. The linkage with oligonucleotides is illustrated by way of example in the formulae XVIII and XIX.



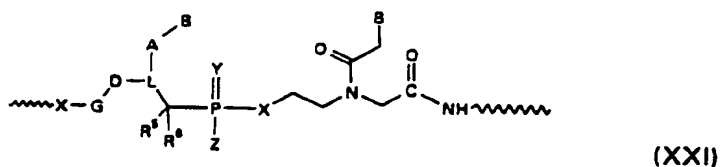
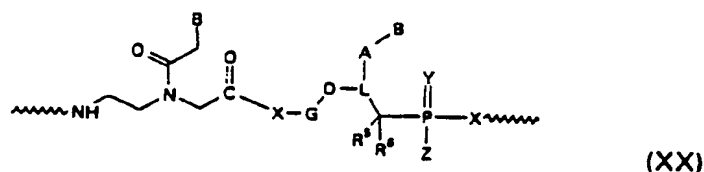
(XVIII)



(XIX)

R^{17} is H, OH, F, 2'-O-(C₁-C₆)-alkyl or 2'-O-(C₂-C₆)alkenyl, preferably H or methoxy or O-allyl, particularly preferably H. All other variables are illustrated above.

Formula XX and formula XXI, in which the variables have the above meaning, illustrate by way of example the linkage with PNAs.



The combinations of the compounds according to the invention utilizing (abbreviated PMENA) oligonucleotides or modified oligonucleotides, will be schematically illustrated again (OLIGO is unmodified or modified oligonucleotides) for example PNAs or other modifications, as are described above:

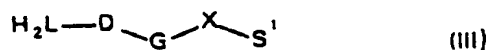
Examples of such combinations are:

- 5'- OLIGO - PMENA
- 5'- PMENA - OLIGO
- 5'- OLIGO - PMENA - OLIGO
- 5'- OLIGO - (PMENA - OLIGO)_a (a = 1-20)
- 5'- PMENA - OLIGO - PMENA
- 5'- PMENA - (OLIGO - PMENA)_a (a = 1-20)

The synthesis of these combined compounds is carried out in such a way that, according to the molecule, the synthesis of the PMENA units, which is described in the following, is begun first, which are then coupled with the oligonucleotide units. In this process, the oligonucleotides are coupled as monomer units or by block condensation by solid-phase synthesis or by solution synthesis by methods known to the person skilled in the art (Sonveaux, Bioorganic Chemistry 14 (1986) 274ff). The condensations are alternatively carried out by the amidite method, the H-phosphonate method or the phosphorotriester process (Sonveaux, Bioorganic Chemistry 14 (1986) 274ff). If, conversely, PMENA units are coupled to OLIGO units, this is preferably carried out by the method described in f₁) below. Conjugation with PNA units is carried out in the same manner or, if (monomeric or oligomeric) PNA units are coupled to PMENA units, with the methods of peptide synthesis or of ester synthesis known to the person skilled in the art. These combinations can be made by means apparent to those skilled in the art in view of the present specification.

The invention furthermore relates to a process for the preparation of compounds of the formula I, which comprises

a₁) reacting a compound of formula III

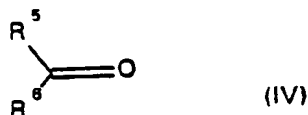


in which

D, G, L and X have the abovementioned meanings and S¹ is a suitable protective group, such as dimethoxytrityl, monomethoxytrityl, trityl, pixyl, tert-

butoxycarbonyl or fluorenylmethoxycarbonyl, preferably monomethoxytrityl or tert-butoxycarbonyl,

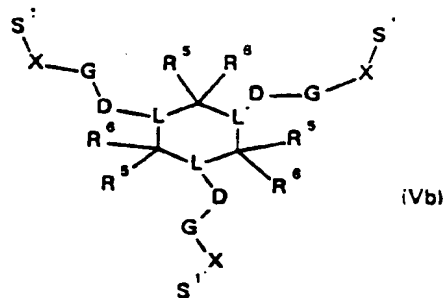
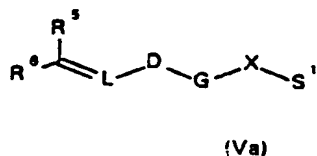
with a compound of formula IV



in which

R^5 and R^6 have the abovementioned meanings,

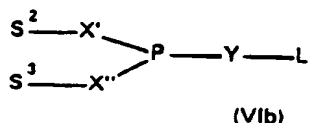
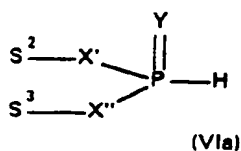
in a suitable organic solvent, for example in methanol, ethanol, isopropanol, butanol, acetonitrile, dichloromethane (DCM), chloroform, benzene, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), diethyl ether, ethyl acetate (EA), tetrahydrofuran (THF), N-methylpyrrolidone, petroleum ether, xylene or toluene or mixtures of suitable solvents, preferably in methanol or ethanol, at temperatures sufficient to form (e.g. from 0°C to 100°C, preferably at 10 to 50°C) compounds of formula Va or Vb



where, in the choice of the reaction conditions which are known to the person skilled in the art (e.g. in S.R. Sandler, W. Karo "Organic Functional Group Preparations", Vol. II, Second Edition, Academic Press, London,

1986, Chapter 12 ("Imines")), care is to be taken that they are compatible with the protective group S^1 , i.e. if, for example, an acid-labile protective group such as the monomethoxytrityl protective group is chosen, no acid should be added during the reaction,

b₁) reacting compounds of the formula Va or Vb with compounds of the formula VIa or VIb, preferably with compounds of the formula VIa



in which

Y is as defined above,

X' and X'' independently of one another have the same meaning as X defined above,

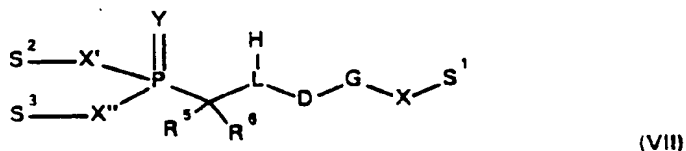
S^2 and S^3 independently of one another are protective groups such as methyl, ethyl, phenyl, 2-chlorophenyl, 2,5-dichlorophenyl, 2,4-dichlorophenyl, 4-nitrophenyl, 4-methoxyphenyl, 2-cyanoethyl, 2-(4-nitrophenyl)ethyl, allyl, benzyl, 2,2,2-trichloro-1,1-dimethylethyl, 4-methoxybenzyl, 2,2,2-trichloroethyl, 8-hydroxyquinoline or other phosphate protective groups, such as are known to the person skilled in the art (Sonveaux, Bioorganic Chemistry 14 (1986) 274 ff), but preferably methyl, ethyl, phenyl, 2-(4-nitrophenyl)ethyl, allyl or 2,2,2-trichloroethyl, and

L^1 is a leaving group, preferably (C₁-C₄)-alkyl,

in a suitable organic solvent, for example in methanol, ethanol, isopropanol, butanol, acetonitrile, benzene, DMF,

DMSO, DCM, EA, chloroform, diethyl ether, THF, N-methylpyrrolidone, petroleum ether, xylene or toluene or mixtures of suitable solvents, preferably in THF, at temperatures sufficient to form a compound of formula VII, e.g. from 0°C to 100°C, preferably at 50 to 80°C, if appropriate with optional addition of bases, such as tri-(C₁-C₆)-alkylamine, N-alkylmorpholine, pyridine, N,N-dimethylaminopyridine, butyllithium, lithium diisopropylamide (LDA), sodium hydride, sodium amide, potassium carbonate, cesium carbonate, potassium tert-butoxide or complex bases such as sodium amide-R¹¹ONa, where R¹¹ is (C₂-C₆)-alkyl or CH₃CH₂-O-CH₂CH₃, or uncharged, peralkylated polyaminophosphazene bases (Schwesinger, Nachr. Chem. Techn. Lab. 38 (1990) 1214; Angew. Chem. 99 (1987) 1212), but preferably without addition of base.

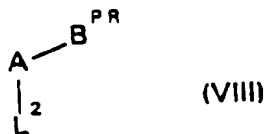
Formula VII has the following formula:



in which

D, G, L, R⁵, R⁶, S¹, S², S³, X, X', X'' and Y are as defined above;

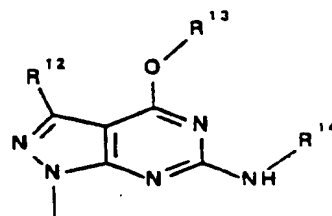
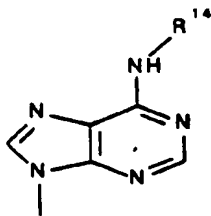
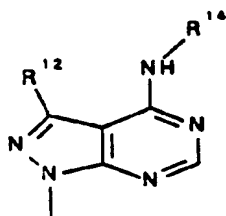
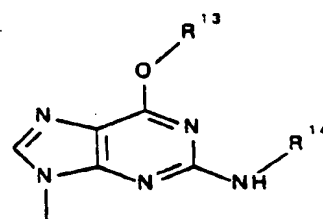
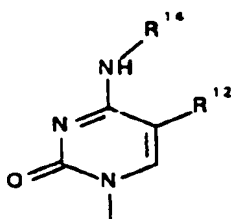
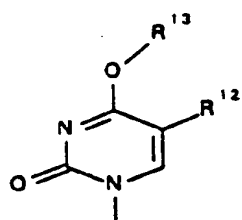
c₁) reacting a compound of formula VII with a compound of formula VIII



whose synthesis is described, for example, in Dueholm et al., J. Org. Chem. 59 (1994) 5767 and in which

A has the abovementioned meaning,

5 B^{PR} has the same meaning as B, but is optionally present in protected form, i.e. if B is a natural or unnatural nucleobase, B^{PR} is the nucleobase whose amino or hydroxyl group's are protected by suitable known protective groups, such as the para-nitrophenylethyl group, the benzoyl group, the allyl group and the para-
 10 (t-butyl)benzoyl group for the hydroxyl group and the acetyl, benzoyl, para-(t-butyl)benzoyl, para-(methoxy)benzoyl group, para-nitrophenylethoxycarbonyl group, isobutyryl group, para-(t-butyl)phenylacetyl group, N,N-dimethylformamidino group,
 15 fluorenylmethyloxycarbonyl group, benzyloxycarbonyl group or phenoxyacetyl group for the amino group or other protective groups customary for nucleobases in oligonucleotide chemistry (Sonveaux, Bioorganic
 20 Chemistry 14 (1986) 274ff; Beaucage, Tetrahedron 49 (1993) 2223ff), preferably the following may be mentioned for B^{PR}:



in which

R¹² is hydrogen, 1-propynyl, 1-butyryl, 1-pentyryl or 1-hexynyl, in particular hydrogen, 1-propynyl or 1-hexynyl; and

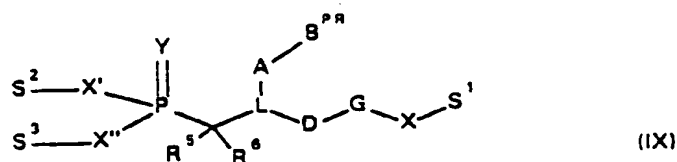
R¹³ is hydrogen, diphenylcarbamoyl or 2-(4-nitrophenyl)-ethyl and

R¹⁴ is acetyl, benzoyl, para-(t-butyl)benzoyl, para-(methoxy)benzoyl, para-nitrophenylethyloxycarbonyl, isobutyryl, para-(t-butyl)phenylacetyl, benzyloxy-carbonyl or phenoxyacetyl, and

L² is a leaving group known to the person skilled in the art, such as Cl, Br, O-SO₂methyl, O-SO₂trifluoromethyl, O-tosylate or O-C₆F₅, or, if A has the meaning of formula IIb, can also be OH;

in a suitable organic solvent, for example in acetonitrile, benzene, DMF, DMSO, DCM, EA, chloroform, diethyl ether, tetramethylurea, THF, N-methylpyrrolidone, petroleum ether, xylene or toluene or mixtures of suitable solvents, preferably in DMF, at temperatures sufficient to form a compound of the formula IX, e.g. from -20°C to 100°C, more preferably at 0 to 50°C, if appropriate with optional addition of bases, such as tri-(C₁-C₆)-alkylamine, N-alkylmorpholine, pyridine, N,N-dimethylaminopyridine, butyllithium, lithium diisopropylamide (LDA), sodium hydride, sodium amide, potassium carbonate, cesium carbonate, potassium tert-butoxide or complex bases such as sodium amide-R¹¹ONa, where R¹¹ is (C₂-C₆)-alkyl or CH₃CH₂-O-CH₂CH₃, or the bases are uncharged, peralkylated polyaminophosphazene bases (Schwesinger, Nachr. Chem. Techn. Lab. 38 (1990) 1214; Angew. Chem. 99 (1987) 1212); and if A is formula IIb and L² is OH, preferably with addition of triethylamine, diisopropylethylamine or N-ethylmorpholine or without addition of base and with addition of a coupling reagent customary for the coupling of peptide bonds,

Formula IX has the following formula:



in which

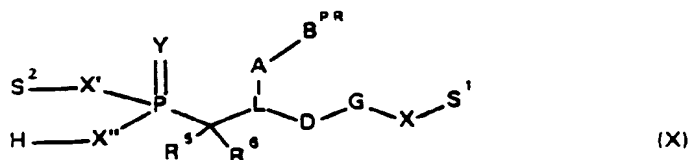
10 A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², S³, X, X', X'' and Y are as defined above:

15 d₁) removing the protective group S³ from compounds of the formula IX by known processes (e.g. Greene, Wuts, Protective Groups in Organic Synthesis, J. Wiley & Sons, New York 1991). For example, for compounds of the formula IX, in which S² and S³ are 2-(4-nitrophenyl)ethyl, by treatment with 0.1M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine or acetonitrile at room temperature or for

20 compounds of the formula IX in which S² and S³ are phenyl or ethyl, by treatment with an aqueous ammonia or for compounds of the formula IX in which S² is 2-(4-nitrophenyl)ethyl and S³ is allyl, by treatment with Pd[P(C₆H₅)₃]₄ and triphenylphosphine in DCM (Hayakawa et al., J. Org. Chem. 58 (1993) 5551), or for compounds of the formula IX in which S² is 2-(4-nitrophenyl)ethyl and S³ is allyl, by treatment with 0.5M DBU in pyridine or acetonitrile or for compounds of the formula IX in which S² is 2-cyanoethyl and S³ is allyl, by treatment with triethylamine in pyridine, or for compounds

30 of the formula IX in which S² is 2-(4-nitrophenyl)ethyl and S³ is 2,2,2-trichloro-1,1-dimethylethyl, by treatment with tributylphosphine,

compounds of the formula X being obtained

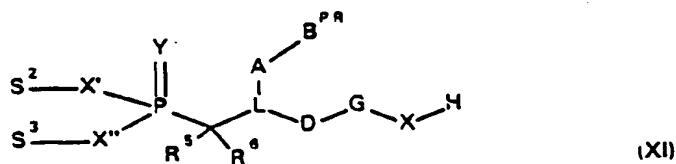


10 in which

A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², X, X', X'' and Y are as defined above;

15 e₁) removing the protective group S¹ from compounds of the formula IX by known processes (e.g. Greene, Wuts, Protective Groups in Organic Synthesis, J. Wiley & Sons, New York 1991, Sonveaux, Bioorganic Chemistry 14 (1986) 274 ff), thus, for example, the monomethoxytrityl protective group is removed by treatment with acid, for example by treatment with 80%
20 acetic acid, with 1-4% dichloroacetic acid in methylene chloride or chloroform, with 2% p-toluenesulfonic acid in DCM/methanol or by treatment with 1% trifluoroacetic acid in chloroform,

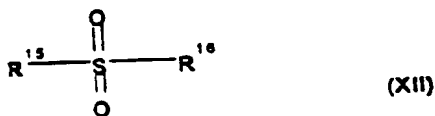
25 compounds of the formula XI being obtained



35 in which

A, B^{PR}, D, G, L, R⁵, R⁶, S², S³, X, X', X'' and Y are as defined above;

f₁) reacting compounds of the formula XI with compounds of the formula X according to the "phosphotriester process" known from oligonucleotide chemistry (Sonveaux, Bioorganic Chemistry 14 (1986) 2744ff, Reese, J. Chem. Soc. Perkin Trans. 1993, 2291ff) in a suitable organic solvent, such as acetonitrile, benzene, DMF, DMSO, DCM, EA, chloroform, diethyl ether, tetramethylurea, THF, N-methylpyrrolidone, petroleum ether, xylene or toluene or mixtures of suitable solvents, preferably in pyridine, at temperatures from -20°C to 100°C, more preferably at 0 to 50°C, with addition of a coupling reagent, such as 6-nitrobenzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (NBOP, Hashmi, Nucleosides & Nucleotides 13 (1994) 1059), benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, B. Castro, J.R. Dormoy, G. Evin and C. Selve, Tetrahedron Lett. 1975, 1219-1222), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, J. Coste, D. Le-Nguyen and B. Castro, Tetrahedron Lett. 1990, 205-208), O-(7-aza)benzotriazol-1-yltetramethyluronium hexafluorophosphate (HATU, L. Carpino, J. Am. Chem. Soc. 1993, 115, 4397), N,N-bis[2-oxo-3-oxazolidinyl]diaminophosphoryl chloride (Katti, Tetrahedron Lett. 26 (1985) 2547), 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphorinane (Stawinski, Nucl. Acids Res., Symp. Ser. 24, 1991, 229) or a compound of the formula XII



in which

R¹⁵ is (C₆-C₁₂)-aryl, optionally substituted one to four times by (C₁-C₆)-alkyl, (C₁-C₆)-alkoxy, nitro, chlorine

or bromine and where one to 3 carbon atoms are optionally substituted by heteroatoms, preferably nitrogen, i.e. for example phenyl, tolyl, 2,4,6-trimethylphenyl, 2,4,6-triisopropylphenyl, 2,3,5,6-tetramethylbenzene (Losse, Liebigs Ann. Chem. 1989, 19ff), 4-bromobenzene, 2-nitrobenzene, 4-nitrobenzene or 8-quinolyl, preferably 2,4,6-trimethylphenyl or 2,4,6-triisopropylphenyl, and

R¹⁶ is a leaving group such as chlorine, bromine, imidazole, triazole, 4-nitroimidazole, 1,2,3,4-tetrazole or 3-nitro-1,2,4-triazole,

preferably using a coupling reagent of the compound of the formula XII or BOP, PyBOP or HATU,

optionally with addition of a catalyst (Reese, J. Chem. Soc. Perkin Trans. 1993, 2291ff), such as N-methylimidazole, pyridine-N-oxides such as 4-methoxypyridine-N-oxide or 4-ethoxypyridine-N-oxide, 4,6-dinitro-1-hydroxybenzotriazole, 1-hydroxy-5-phenyltetrazole, 1-hydroxy-5-(4-nitrophenyl)tetrazole, 3-nitro-1H-1,2,4-triazole, 5-(3-nitrophenyl)-1H-tetrazole, 5-(3,5-dinitrophenyl)-1H-tetrazole, 5-(1-methylimidazole-2-yl)-1H-tetrazole, 5-[(1-methylimidazole-2-yl)methyl]-1H-tetrazole or 1-hydroxy-4-nitro-6-(trifluoromethyl)benzotriazole, preferably with 4-ethoxypyridine-N-oxide or 4-methoxypyridine-N-oxide as a catalyst,

where the preparation of the coupling reagents can be carried out in situ, or else carried out separately and the solution of the activated species (compound of formula (X) in combination with a coupling reagent) can be added in a suitable solvent,



g₁) starting from compounds of the formula XIII, repeating the steps e₁) and f₁) up to the desired chain length, compounds of the formula XIV resulting



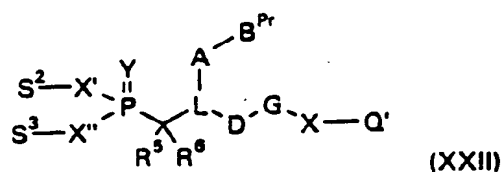
h₁) removing the protective groups S¹, S² and S³ and the protective groups on B^{PR} according to known processes (e.g. Greene, Wuts, Protective Groups in Organic Synthesis, J. Wiley & Sons, New York 1991),

i.e., for example, the protective group S^1 as described in step e_1), the protective groups S^2 or S^3 , if they are 2-(4-nitrophenyl)ethyl, by treatment with 0.5M 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pyridine or acetonitrile at room temperature, if S^2 or S^3 is phenyl, by treatment

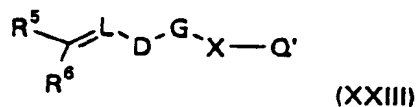
with aqueous ammonia, if S^2 or S^3 is allyl, by treatment with $\text{Pd}[\text{P}(\text{C}_6\text{H}_5)_3]_4$ and triphenylphosphine in DCM (Hayakawa et al., J. Org. Chem. 58 (1993) 5551), if S^2 or S^3 is 2-cyanoethyl, by treatment with triethylamine in pyridine, or if S^2 or S^3 is 2,2,2-trichloro-1,1-dimethylethyl, by treatment with tributylphosphine, and the protective groups on B^{PR} , for example if R^{14} is para-nitrophenylethyloxycarbonyl, with 0.5M DBU in pyridine, if R^{14} is isobutyryl or benzoyl or para-methoxybenzoyl, with conc. NH_4OH at 20 to 60°C or, if R^{13} is 2-(4-nitrophenyl)ethyl, by treatment with 0.5M DBU in pyridine or acetonitrile, is preferred, if S^1 equals monomethoxytrityl and S^2 equals 2-(para-nitrophenyl)ethyl, preferably the monomethoxytrityl group is first removed as described in e_1 , then S^2 is removed as described, then the remaining protective groups, for example on the nucleobases, are removed;

and optionally introducing the groups Q and Q' according to processes known to the person skilled in the art, (see, for example, Uhlmann & Peyman, Chem. Rev. 90 (1990) 543; M. Manoharan in "Antisense Research and Applications", Crooke and Lebleu, Eds., CRC Press, Boca Raton, 1993, Chapter 17, p.303ff; EP-A 0 552 766; S. Agrawal in Methods in Molecular Biology, Vol. 26, P. 93 ff, Humana Press, Totowa 1994), and optionally cyclizing the compounds obtained according to Wang, Nucl. Acids Res. 22 (1994) 2326, whereby compounds of the formula I result.

Alternatively, conjugates Q' can also be incorporated into the monomer units of the formula XXII by processes known to the person skilled in the art (J. March, "Advanced Organic Chemistry", Fourth Ed., J. Wiley & Sons, 1992), which are then incorporated into the compounds of the formula I according to the processes mentioned.

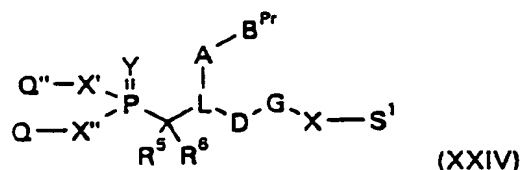


Compounds of the formula XXII can be prepared, for example, for Q' = alkyl, by reaction of compounds of the formula XXIII with compounds of the formula VIA or VIB and further reactions analogously to those described for the formulae Va and Vb.



Compounds of the formula XXII can also be prepared from compounds of the formula IX by removal of the protective group S¹ and introduction of the group Q' according to known processes (J. March, "Advanced Organic Chemistry", Fourth Ed., J. Wiley & Sons, 1992).

Alternatively, conjugates Q and Q" can also be incorporated into the monomer units of the formula XXIV by processes known to the person skilled in the art (J. March, "Advanced Organic Chemistry", Fourth Ed., J. Wiley & Sons, 1992), which are then incorporated into the compounds of the formula I according to the processes mentioned.



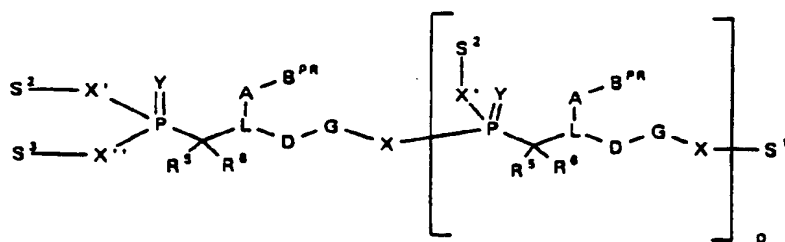
Coupling reagents for the linkage of peptide bonds (see c₁) are described, for example, in Houben-Weyl, Methoden der organischen Chemie, [Methods of organic chemistry] Volume 15/2, Georg Thieme Verlag Stuttgart 1974 and further reagents such as BOP (B. Castro, J.R. Dormoy, G. Evin and C. Selve, Tetrahedron Lett. 1975, 1219-1222), PyBOP (J. Coste, D. Le-Nguyen and B. Castro, Tetrahedron Lett. 1990, 205-208), BroP (J. Coste, M.-N. Dufour, A. Pantaloni and B. Castro, Tetrahedron Lett. 1990, 669-672), PyBroP (J. Coste, E. Frerot, P. Jouin and B. Castro, Tetrahedron Lett. 1991, 1967-1970) and uronium reagents, such as HBTU (V. Dourtoglou, B. Gross, V. Lambropoulou, C. Zioudrou, Synthesis 1984, 572-574), TBTU, TPTU, TSTU, TNTU (R. Knorr, A. Trzeciak, W. Bannwarth and D. Gillessen, Tetrahedron Letters 1989, 1927-1930), TOTU (EP-A-O 460 446), HATU (L.A. Carpino, J. Am. Chem. Soc. 1993, 115, 4397-4398), HAPyU, TAPipU (A. Ehrlich, S. Rothmund, M. Brudel, M. Beyermann, L.A. Carpino and M. Bienert, Tetrahedron Lett. 1993, 4781-4784), BOI (K. Akaji, N. Kuriyama, T. Kimura, Y. Fujiwara and Y. Kiso, Tetrahedron Lett. 1992, 3177-3180) or acid chlorides or acid fluorides (L. A. Carpino, H. G. Chao, M. Beyermann and M. Bienert, J. Org. Chem., 56 (1991), 2635; J.-N. Bertho, A. Loffet, C. Pinel, F. Reuther and G. Sennyey in E. Giralt and D. Andreu (Eds.) Peptides 1990, Escom Science Publishers B. V. 1991, pp. 53-54; J. Green and K. Bradley, Tetrahedron 1993, 4141-4146), 2,4,6-

mesitylenesulfonyl-3-nitro-1,2,4-triazolide (MSNT) (B. Blankemeyer-Menge, M. Nimitz and R. Frank, Tetrahedron Lett. 1990, 1701-1704), 2,5-diphenyl-2,3-dihydro-3-oxo-4-hydroxythiophene dioxide (TDO) (R. Kirstgen, R.C. Sheppard, W. Steglich, J. Chem. Soc. Chem. Commun. 1987, 1870-1871) or activated esters (D. Hudson, Peptide Res. 1990, 51-55) in the respective references.

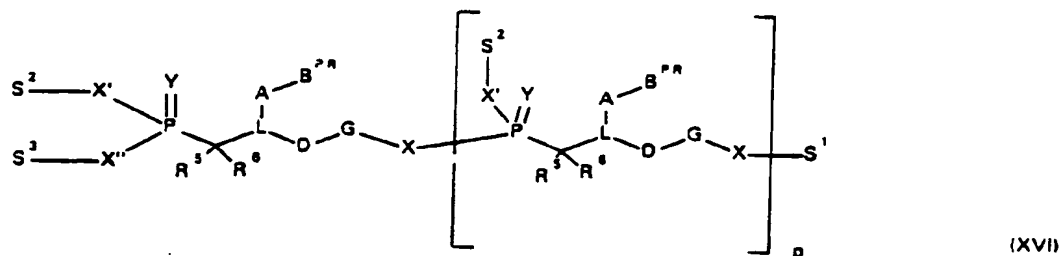
The use of carbodiimides, e.g. dicyclohexylcarbodiimide or diisopropylcarbodiimide, is further preferred. Phosphonium reagents, such as PyBOP or PyBroP, uronium reagents, such as HBTU, TBTU, TPTU, TSTU, TNTU, TOTU or HATU and BOI are also preferably used.

In this case, the coupling can be carried out directly by addition of compounds of the formula VIII using the activating reagent and optionally with addition of additives such as 1-hydroxybenzotriazole (HOBt) (W. König, R. Geiger, Chem. Ber. 103, 788 (1970)) or 3-hydroxy-4-oxo-3,4-dihydrobenzotriazine (HOObt) (W. König, R. Geiger, Chem. Ber. 103, 2034 (1970)) or else the preactivation of the units as an activated ester can be carried out separately and the solution of the activated species in a suitable solvent can be added.

The invention furthermore relates to a process for the preparation of the compounds of the formula I, in which n is 1 to 100, which comprises, in the compounds of the formulae XV and XVI



(XV)



in which

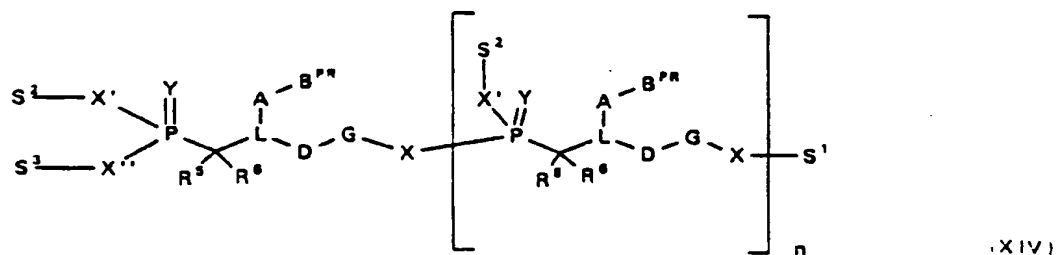
A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², S³, X, X', X'' and Y are as defined above,

o and p independently of one another are zero to 50, preferably zero to 20 and o + p + 1 = n;

a₂) in the compounds of the formula XV removing the protective group S¹ as described under e₁),

b₂) in the compounds of the formula XVI removing the protective group S³ as described under d₁) and

c₂) coupling the resulting compounds with one another as described under f₁), compounds of the formula XIV resulting



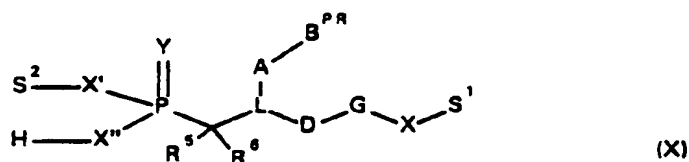
in which

A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², S³, X, X', X'', Y and n are as defined above,

d₂) and reacting these as described under h₁) to give compounds of the formula I.

The invention furthermore relates to a process for the preparation of the compounds of the formula I, which comprises

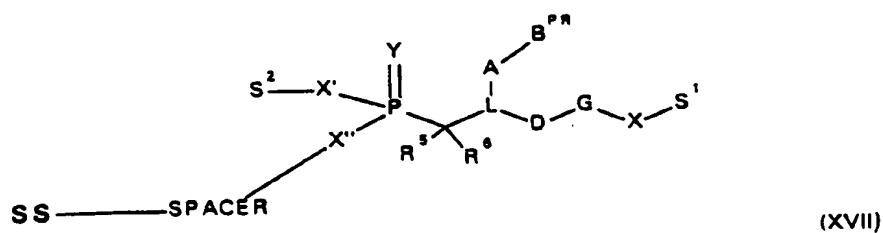
a₃) coupling compounds of the formula X



in which

A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², X, X', X'' and Y are as defined above,

to a solid support via a SPACER according to known processes, to give compounds of the formula XVII,



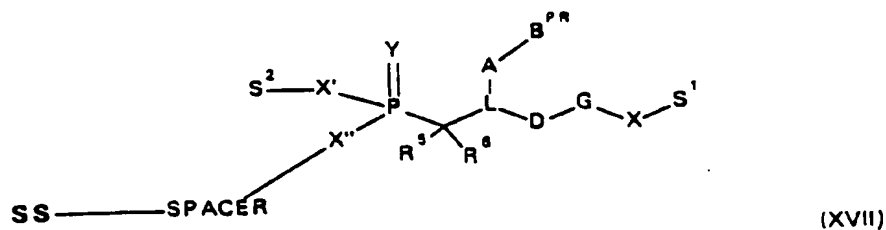
in which

A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², X, X', X'' and Y are as defined above,

SS is a solid support suitable for solid-phase synthesis, such as aminopropyl-CPG (CPG="Controlled Pore Glass) or Tentagel, and

SPACER is a group removable from the support after synthesis has taken place, such as is known to the person skilled in the art (Sonveaux, Bioorganic Chemistry 14 (1986) 274ff), for example the bis(hydroxyethyl)sulfonyl group, as is described in EP-A 0 552 766 (HOE 92/F012), or SPACER is bifunctional conjugate molecules Q, which are linked to the solid support via known removable groups, for example is nucleotides or oligonucleotides which are bound to the solid support via a succinic acid radical (Sonveaux, Bioorganic Chemistry 14 (1986) 274ff) or poly- or oligoethylene glycols which are bound to the solid support via a succinic acid radical (Jäschke, Tetrahedron Lett. 34 (1993) 301) or, for example, cholesterol derivatives which are bound to the solid support via a succinic acid radical (MacKellar, Nucl. Acids Res. 20 (1992) 3411);

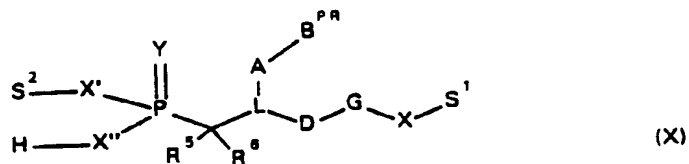
b₃) removing the protective group S¹ from compounds of the formula XVII



in which

A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², SS, SPACER, X, X', X'' and Y are as defined above, as described under e₁);

c₃) reacting the resulting compound with compounds of the formula X



in which

A, B^{PR}, D, G, L, R⁵, R⁶, S¹, S², X, X', X'' and Y are as defined above,
as described under f₁);

d₃) repeating the steps b₃) and c₃) up to the desired chain length;

e₃) optionally coupling conjugates Q' by known processes (see, for example, Uhlmann & Peyman, Chem. Rev. 90 (1990) 543; M. Manoharan in "Antisense Research and Applications", Crooke and Lebleu, Eds., CRC Press, Boca Raton, 1993, Chapter 17, P.303ff; EP-A 0 552 766; S. Agrawal in "Methods in Molecular Biology", Vol. 26, P. 93ff, Humana Press, Totowa 1994);

f₃) removing the compounds produced in this way from the solid support by known processes, for example the bis(hydroxyethyl)sulfonyl linkers, as described in EP-A 0 552 766, (HOE92/F012) by treatment with DBU, the succinic acid linker by treatment with aqueous ammonia and the protective groups as described in step h₁), where the removal of the protective groups can also be carried out before the cleavage from the support.

The compounds of the formula I are used as inhibitors of gene expression. The invention therefore further relates to the use of therapeutically active compounds according to the

invention for the production of a pharmaceutical and to a process for the production of a pharmaceutical which comprises mixing the compounds according to the invention with a physiologically acceptable excipient and also, if appropriate, suitable additives and/or auxiliaries.

Therapeutically active compounds are understood in general as meaning those which, on account of the sequence of the units B which correspond to the nucleobases, carry out a function as analogs of antisense oligonucleotides, triple helix-forming oligonucleotides, aptamers (RNA or DNA molecules which can bind to specific target molecules, e.g. proteins or receptors (e.g. L.C. Bock et al., Nature 1992, 355, 564) or ribozymes (catalytic RNA, see for example, Castanetto et al., Critical Rev. Eukar. Gene Expr. 1992, 2, 331), in particular as analogs of antisense oligonucleotides and triple helix-forming oligonucleotides.

Moreover, the present invention further relates to the use of the compounds according to the invention as a diagnostic, for example for the detection of the presence or absence or the amount of a specific double-stranded or single-stranded nucleic acid molecule in a biological sample.

For use according to the invention, the compounds according to the invention generally have a length (n-1) of about 6 - 100, preferably of about 10 - 40, in particular of about 12 - 31 nucleotides. Otherwise, the preferred ranges, modifications or conjugations described above also apply here.

The pharmaceuticals of the present invention can be used, for example, for the treatment of illnesses which are caused by viruses, for example by HIV, HSV-1, HSV-2, influenza, VSV, hepatitis B or papilloma viruses.

a) against HIV, e.g.

	ACACCCAATTCTGAAAATGG	SEQ ID NO:1
	AGGTCCTGTTCGGGCGCCA	SEQ ID NO:2
	GGTCCCTGTTCGGGCGCCA	SEQ ID NO:26
5	GTCGACACCCAATTCTGAAAATGGATAA	SEQ ID NO:3
	GCTATGTGCGACACCCAATTCTGAAA	SEQ ID NO:4
	GTCGCTGTCTCCGCTTCTTCTTCCTG	SEQ ID NO:5
	GTCTCCGCTTCTTCTTCCTGCCATAGG	SEQ ID NO:6

b) against HSV-1, e.g.

	GCGGGGCTCCATGGGGGTCG	SEQ ID NO:7
	GGAGGATGCTGAGGAGG	SEQ ID NO:28
	GGAGGATGCTGAGG	SEQ ID NO:29
15	CAGGAGGATGCTGAGGAGG	SEQ ID NO:30

The pharmaceuticals of the present invention are also suitable, for example, for the treatment of cancer or of restenosis. For example, in this case sequences (base sequences) can be used which are directed against targets which are responsible for carcinogenesis or cancer growth. Such targets are, for example:

1) Nuclear oncoproteins such as c-myc, N-myc, c-myb, c-fos, c-fos/jun, PCNA, p120

2) Cytoplasmic/membrane-associated oncoproteins such as EJ-ras, c-Ha-ras, N-ras, rrg, bcl-2, cdc-2, c-raf-1, c-mos, c-src, c-abl

3) Cellular receptors such as EGF receptor, c-erbA, retinoid receptors, protein kinase regulatory subunits, c-fms

4) Cytokines, growth factors, extracellular matrix such as CSF-1, IL-6, IL-1a, IL-1b, IL-2, IL-4, bFGF, myeloblastin, fibronectin.

4) Cytokines, growth factors, extracellular matrix such as CSF-1, IL-6, IL-1a, IL-1b, IL-2, IL-4, bFGF, myeloblastin, fibronectin.

5 Sequences according to the invention (base sequences) which are active against targets of this type are, for example,

a) against c-Ha-ras, e.g.

10 CAGCTGCAACCCAGC SEQ ID NO:8

c) c-myc, e.g.

15 GGCTGCTGGAGCGGGGCACAC SEQ ID NO:9
AACGTTGAGGGGCAT SEQ ID NO:10

d) c-myb, e.g.

20 GTGCCGGGGTCTTCGGGC SEQ ID NO:11
GTGCCGGGGTCTTCGGG SEQ ID NO:27

e) c-fos, e.g.

25 GGAGAACATCATGGTCAAG SEQ ID NO:12
CCCGAGAACATCATGGTCAAG SEQ ID NO:13
GGGGAAAGCCCGGCAAGGGG SEQ ID NO:14

f) p120, e.g.

30 CACCCGCCTTGGCCTCCCAC SEQ ID NO:15

g) EGF receptor, e.g.

35 GGGACTCCGGCGAGCGC SEQ ID NO:16
GGCAAACCTTTCTTTTCCTCC SEQ ID NO:17

h) p53 tumor suppressor, e.g.

GGGAAGGAGGAGGATGAGG

SEQ ID NO:18

GGCAGTCATCCAGCTTCGGAG

SEQ ID NO:19

5

i) bFGF, e.g.

GGCTGCCATGGTCCC

SEQ ID NO:31

10

The pharmaceuticals of the present invention are further suitable, for example, for the treatment of illnesses which are affected by integrins or cell-cell adhesion receptors, for example by VLA-4, VLA-2, ICAM or ELAM.

15

Sequences according to the invention (base sequences) which are active against targets of this type are, for example

a) VLA-4, e.g.

20

GCAGTAAGCATCCATATC

SEQ ID NO:20

b) ICAM, e.g.

CCCCCACCCTTCCCCTCTC

SEQ ID NO:21

25

CTCCCCCACCCTTCCCCTC

SEQ ID NO:22

GCTGGGAGCCATAGCGAGG

SEQ ID NO:23

c) ELAM-1, e.g.

30

ACTGCTGCCTCTTGTCTCAGG

SEQ ID NO:24

CAATCAATGACTTCAAGAGTTC

SEQ ID NO:25

35

The pharmaceuticals of the present invention are further suitable, for example, for the treatment of illnesses which are induced by factors such as TNF alpha.

Sequences according to the invention (base sequences) which are active against targets of this type are, for example

a) TNF-alpha, e.g.

5

TCATGGTGTCTTTGCAGCC

SEQ ID NO:32

TCATGGTGTCTTTGCAG

SEQ ID NO:33

10

The pharmaceuticals can be used, for example, in the form of pharmaceutical preparations which can be administered, for example, topically or orally, e.g. in the form of tablets, coated tablets, hard or soft gelatin capsules, solutions, emulsions or suspensions. They can also be administered rectally, e.g. in the form of suppositories, or

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parenterally, e.g. in the form of injection solutions. For the production of pharmaceutical preparations, these compounds can be processed in therapeutically inert organic and inorganic excipients. Examples of such excipients for tablets, coated tablets and hard gelatin capsules are

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lactose, maize starch or derivatives thereof, talc and stearic acid or salts thereof. Suitable excipients for the production of solutions are water, polyols, sucrose, invert sugar and glucose. Suitable excipients for injection solutions are water, alcohols, polyols, glycerol and

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vegetable oils. Suitable excipients for suppositories are vegetable and hardened oils, waxes, fats and semiliquid polyols. The pharmaceutical preparations can also contain preservatives, solvents, stabilizers, wetting agents,

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emulsifiers, sweeteners, colorants, flavorings, salts for changing the osmotic pressure, buffers, coating compositions, antioxidants, and also, if appropriate, other therapeutic active compounds.

35

A preferred administration is oral administration. A further preferred form of administration is injection. For this, the antisense oligonucleotides are formulated in a liquid

5 solution, preferably in a physiologically acceptable buffer, such as Hank's solution or Ringer's solution. The therapeutically active compounds according to the invention, however, can also be formulated in solid form and dissolved or suspended before use. The preferred dosages for systemic administration are about 0.01 mg/kg to about 50 mg/kg of body weight per day.

10 List of sequences:

ACACCCAATTCTGAAAATGG

SEQ ID NO:1

AGGTCCCTGTTCTGGGCGCCA

SEQ ID NO:2

GTCGACACCCAATTCTGAAAATGGATAA

SEQ ID NO:3

15

GCTATGTCGACACCCAATTCTGAAA	SEQ ID NO:4
GTCGCTGTCTCCGCTTCTTCTTCCTG	SEQ ID NO:5
GTCTCCGCTTCTTCTTCCTGCCATAGG	SEQ ID NO:6
GCGGGGCTCCATGGGGGTCG	SEQ ID NO:7
CAGCTGCAACCCAGC	SEQ ID NO:8
GGCTGCTGGAGCGGGGCACAC	SEQ ID NO:9
AACGTTGAGGGGCAT	SEQ ID NO:10
GTGCCGGGGTCTTCGGGC	SEQ ID NO:11
GGAGAACATCATGGTCGAAAG	SEQ ID NO:12
CCCGAGAACATCATGGTCGAAAG	SEQ ID NO:13
GGGGAAAGCCCGGCAAGGGG	SEQ ID NO:14
CACCCGCCTTGGCCTCCCAC	SEQ ID NO:15
GGGACTCCGGCGCAGCGC	SEQ ID NO:16
GGCAAACCTTTCTTTTCCTCC	SEQ ID NO:17
GGGAAGGAGGAGGATGAGG	SEQ ID NO:18
GGCAGTCATCCAGCTTCGGAG	SEQ ID NO:19
GCAGTAAGCATCCATATC	SEQ ID NO:20
CCCCCACCACCTCCCCTCTC	SEQ ID NO:21
CTCCCCCACCACCTCCCCTC	SEQ ID NO:22
GCTGGGAGCCATAGCGAGG	SEQ ID NO:23
ACTGCTGCCTCTTGTCTCAGG	SEQ ID NO:24
CAATCAATGACTTCAAGAGTTC	SEQ ID NO:25
GGTCCCTGTTGCGGCGCCA	SEQ ID NO:26
GTGCCGGGGTCTTCGGG	SEQ ID NO:27
GGAGGATGCTGAGGAGG	SEQ ID NO:28
GGAGGATGCTGAGG	SEQ ID NO:29
CAGGAGGATGCTGAGGAGG	SEQ ID NO:30
GGCTGCCATGGTCCC	SEQ ID NO:31
TCATGGTGTCTTTGCAGCC	SEQ ID NO:32
TCATGGTGTCTTTGCAG	SEQ ID NO:33

Examples:

1) Di(2-(p-nitrophenyl)ethyl) N-(4-methoxytriphenyl-methoxy)ethylaminomethanephosphonate

5

1a) N-Fluorenylmethoxycarbonyl-2-aminoethanol

10

8.61 g (0.141 mol) of 2-aminoethanol were dissolved in 250 ml of dioxane and 150 ml of H₂O. At 15-20°C, 17.79 g (0.212 mol) of NaHCO₃ were first added, then 50 g (0.148 mol) of fluorenylmethoxycarbonyl-N-succinimide in portions. The mixture was stirred at room temperature for 1h, then it was evaporated to dryness. The residue was partitioned between dichloromethane (DCM) and H₂O, the organic phase was dried over Na₂SO₄ and the solvent was evaporated in vacuo. The residue was stirred with 100 ml of ether, and the product was filtered off with suction and washed well with ether. The yield was 38.77 g (97%).

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20

MS (ES⁺): 284.2 (M+H)⁺; ¹H-NMR (200 MHz, DMSO, TMS):
 δ = 3.05 (dd, 2H, CH₂OH); 3.39 (dd, 2H, N=CH₂); 4.25 (m, 3H, Ar-CH-CH₂); 4.61 (t, 1H, OH); 7.14-7.98 (m, 15H, Ar-H, NH).

25

1b) N-Fluorenylmethoxycarbonyl-2-amino-1-(4-methoxytriphenylmethoxy)ethane

30

10 g (35.3 mmol) of N-fluorenylmethoxycarbonyl-2-aminoethanol (from Example 1a), dissolved in 100 ml of absol. N,N-dimethylformamide (DMF), were treated at 0°C with 5.93 g (45.93 mmol) of diisopropylethylamine (DIPEA) and 10.91 g (35.3 mmol) of 4-methoxytriphenylmethyl chloride and stirred first at 0°C for 1 h, then at room temperature for 1 h. The reaction mixture was evaporated and partitioned between DCM and a saturated aqueous NaHCO₃ solution. The organic phase was washed with H₂O and dried over Na₂SO₄, and the solvent was evaporated in vacuo. To purify the product, it was

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chromatographed on silica gel (first n-heptane/ethyl acetate (EA)/triethylamine (TEA) 70:29:1; then EA/TEA 99:1). The yield was 14.4 g (73%).

5 MS (FAB): 562.3 (M+Li)⁺; ¹H-NMR (200 MHz, DMSO, TMS):
 δ = 2.95 (t, 2H, CH₂O-MMTTr); 3.21 (dd, 2H, N-CH₂); 3.75 (s, 3H, OCH₃); 4.25 (m, 3H, Ar-CH-CH₂); 4.61 (t, 1H, OH); 6.80-7.96 (m, 23H, Ar-H, NH).

10 1c) 2-Amino-1-(4-methoxytriphenylmethoxy)ethane

15 5.0 g (9 mmol) of N-fluorenylmethoxycarbonyl-2-amino-1-(4-methoxytriphenylmethoxy)ethane (from Example 1b), dissolved in 50 ml of absol. DMF, were treated at room temperature with 6.55 g (90 mmol) of diethylamine and the mixture was stirred for 2h. To purify it, it was chromatographed on silica gel (first n-heptane/EA/TEA 50:49:1; then EA/methanol/TEA 79:20:1). The yield was 2.96 g (98.7%).

20 MS (ES⁺): 340.3 (M+Li)⁺; ¹H-NMR (200 MHz, DMSO, TMS):
 δ = 2.75 (t, 2H, CH₂O-MMTTr); 2.93 (dd, 2H, N-CH₂); 3.75 (s, 3H, OCH₃); 6.83-7.47 (m, 14H, Ar-H).

25 1d) 2-Methylimino-1-(4-methoxytriphenylmethoxy)ethane
 (trimer)

30 2.96 g (8.9 mmol) of 2-amino-1-(4-methoxytriphenylmethoxy)ethane (from Example 1c), dissolved in 10 ml of methanol were treated with ice cooling with 1.08g (13.22 mmol) of 37% formaldehyde and stirred at room temperature for 4h, a viscous precipitate being formed. The reaction mixture was evaporated and the residue was chromatographed on silica gel to purify it (n-heptane/EA/TEA 50:49:1). The yield was 1.7 g (55%).

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MS (FAB): 1042.8 (M+Li)⁺; 1034.8 (M-H)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 2.60 (t, 6H, O-CH₂); 2.99 (t, 6H, N-CH₂); 3.69 (s, 9H, OCH₃); 6.78-7.42 (m, 42H, Ar-H).

5 1e) Di(2-(4-nitrophenyl)ethyl) phosphite

23.42 g (0.1 mol) of diphenyl phosphite were heated at 100°C for 14h under argon together with 33.43g (0.2 mol) of p-nitrophenylethanol, and to purify it the mixture was chromatographed on silica gel (n-heptane/EA 50:50; then EA/methanol 80:20). Yield: 55%.

15 MS (FAB): 403.1 (M+Na)⁺; 381.1 (M+H)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 3.03 (t, 4H, Ar-CH₂); 4.20 (4H, dt, O-CH₂); 6.71 (d, J=140Hz; 1H, PH); 7.52 (d, 4H, Ar-H); 8.17 (d, 4H, Ar-H).

20 1f) Di-(2-(p-nitrophenyl)ethyl N-(4-methoxytriphenyl-methoxy)ethylaminomethanephosphonate

341 mg (0.329 mmol) of 2-methylimino-1-(4-methoxytriphenylmethoxy)ethane (trimer) (from Example 1d) were added to 500 mg (1.32 mmol) of di(2-(4-nitrophenyl)ethyl) phosphite (from Example 1e), dissolved in 2 ml of absol. Tetrahydrofuran (THF), and the mixture was stirred at 80°C for 3 h. The solvent was evaporated and the residue was stirred at 100°C for a further 30 min. To purify it, it was chromatographed on silica gel (first EA/TEA 99:1; then EA/methanol/TEA 90:9:1). Yield: 83%.

30 MS (FAB): 732.3 (M+Li)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 2.64-3.06 (m, 10H, Ar-CH₂ + P-CH₂ + CH₂-OMMTr + N-CH₂); 3.73 (s, 3H, OCH₃); 4.16 (dt, 4H, PO-CH₂); 6.78-8.08 (m, 22H, Ar-H).

35

2) Di(2-(p-nitrophenyl)ethyl) N-(N⁶-Anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate

5 2a) 0.952g (8.27 mmol) of N-ethylmorpholine (NEM), 0.834g (2.76 mmol) of (N⁶-anisoyl)cytosin-1-yl-acetic acid and 1.153 g (3.03 mmol) of O-(7-aza)benzotriazol-1-yltetramethyluronium hexafluorophosphate (HATU, L. Carpino, J. Am. Chem. Soc. 1993, 115, 4397) was added to 2.00 g (2.76 mmol) of di(2-(p-nitrophenyl)ethyl) N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (from Example 1f),
10 dissolved in 60 ml of absol. DMF, and the mixture was stirred at room temperature for 12h. The same amount of HATU was then added once again and the mixture stirred at room
15 temperature for a further 3h. To purify it, it was chromatographed on silica gel (DCM/methanol/TEA 95:4:1). The yield was 2.7 g (97%).

MS (ES⁺): 1012.0 (M+H)⁺. ¹H-NMR (200 MHz, DMSO, TMS):
20 δ = 2.94 (t, 4H, P-O-CH₂-CH₂-Ar); 3.06 (t, 2H, MMTr-O-CH₂); 3.23-3.63 (m, 4H, P-CH₂ + N-CH₂); 3.75 (s, 3H, OCH₃); 3.83 (s, 3H, OCH₃); 4.10 (dt, 4H, P-O-CH₂); 4.79 (s, broad, 2H, CO-CH₂); 6.80-8.18 (m, 28H, Ar-H, cytosinyl-H); 11.03 (s, broad, 1H, NH).

25 2b) Mixture as in Example 2a, but using O-(cyano(ethoxycarbonyl)methylenamino)-1,1,3,3-tetramethyluronium tetrafluoroborate (TOTU, EP 0460446) instead of HATU. The yield was 57%. Spectroscopic data: see Example 2a.

30 3) N-(N⁶-Anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid (2-(p-nitrophenyl)ethyl) monoester (triethylammonium salt)

35 1 g (0.99 mmol) of di(2-(p-nitrophenyl)ethyl) N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-

ethylaminomethanephosphonate (from Example 2) was dissolved in 20ml of a 0.1 M solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in absol. acetonitrile and the mixture was stirred at room temperature for 4h. The reaction mixture was partitioned between DCM and an aqueous KH_2PO_4 solution (pH 7), the organic phase was dried over Na_2SO_4 and the solvent was evaporated in vacuo. To purify the residue, it was chromatographed on silica gel (EA/methanol/TEA 70:29:1). The yield was 540 mg (57%).

MS (FAB): 906.5 ($\text{M}-\text{H}+2\text{Na}$)⁺; 884.6 ($\text{M}+\text{Na}$)⁺; 862.5 ($\text{M}+\text{H}$)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 3.00 (m, 4H, P-O-CH₂-CH₂-Ar + MMTr-O-CH₂); 3.38-3.60 (m, 4H, P-CH₂ + N-CH₂); 3.73 (s, 3H, OCH₃); 3.82 (s, 3H, OCH₃); 4.01 (dt, 2H, P-O-CH₂); 4.79 & 5.03 (in each case s, broad, 2H, CO-CH₂); 6.78-8.20 (m, 24H, Ar-H, cytosinyl-H); 11.00 (s broad, 1H, NH).

4) Di(2-(p-nitrophenyl)ethyl) N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(2-hydroxy)ethylamino-methanephosphonate

1.00 g (0.99 mmol) of di(2-(p-nitrophenyl)ethyl) N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonate (from Example 2) was dissolved in 80 ml of 80% aqueous acetic acid and the solution was stirred at room temperature for 4h. The solvent was evaporated and the residue was coevaporated twice with toluene. To purify it, it was chromatographed on silica gel (EA/methanol/TEA 85:14:1). The yield was 522 mg (71%).

MS (FAB): 761.2 ($\text{M}+\text{Na}$)⁺; 739.3 ($\text{M}+\text{H}$)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 2.98 (t, 4H, P-O-CH₂-CH₂-Ar) 3.38-3.67 (m, 4H, N-CH₂CH₂-OH); 3.80-3.89 (m, 2H, P-CH₂); 3.91 (s, 3H, OCH₃); 4.12 (dt, 4H, P-O-CH₂); 4.78 & 4.87 (in each case s, broad, 2H, CO-CH₂); 6.98-8.19 (m, 14H, Ar-H, cytosinyl-H); 11.02 (s, broad, 1H, NH).

5) 5'-MMTr-C^{An}-(P(ONPE)-C^{An}-P(ONPE))₂

The synthesis was carried out analogously to Example 7 from N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenyl-methoxy)ethylaminomethanephosphonic acid (2-(p-nitrophenyl)ethyl) monoester (triethylammonium salt) (Example 3) and di(2-(p-nitrophenyl)ethyl) N-(N⁶-anisoyl) cytosin-1-yl-acetyl-N-(2-hydroxy)ethylaminomethanephosphonate (Example 4). To purify it, the compound was chromatographed on silica gel (EA/methanol/TEA 85/14/1). Yield: 73%.

MS (FAB): 1605 (M+Na)⁺; 1583 (M+H)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 2.94-3.18 (m, 6H, P-O-CH₂-CH₂-Ar) 3.26-3.95 (m, 10H); 3.75 (s, 3H, OCH₃); 3.85 (s, 6H, OCH₃); 3.99-4.36 (m, 8H, P-O-CH₂); 4.75-4.92 (m, broad, 4H, CO-CH₂); 6.83-8.18 (m, 38H, Ar-H, cytosinyl-H); 10.98 & 11.03 (in each case s, broad, 2H, NH).

6) 5'-HO-C^{An}-P(ONPE)-C^{An}-P(ONPE))₂

The synthesis was carried out analogously to Example 4 from "5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE))₂" (Example 5). To purify it, the compound was chromatographed on silica gel (EA/methanol/TEA 85:14:1). The yield was 74%.

MS (FAB): 1332.4 (M+Na)⁺; 1310.3 (M+H)⁺;

7) Diethyl N-(4-methoxytriphenylmethoxy)ethylamino-methanephosphonate

The synthesis was carried out analogously to Example 1f, but using diethyl phosphite. Yield: 87.5%.

MS (FAB): 490.2 (M+Li)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 1.22 (t, 6H, CH₂-CH₃); 2.80 (t, 2H, N-CH₂); 2.91 (d, J =

12.5 Hz, 2H, P-CH₂); 3.02 (t, 2H, CH₂-OMMTr); 3.75 (s, 3H, OCH₃); 4.01 (dq, 4H, PO-CH₂); 6.84-7.45 (m, 14H, Ar-H).

8) Diethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenyl-methoxy)ethylaminomethanephosphonate

570.3 mg (4.22 mmol) of hydroxybenzotriazole (HOBT), 972.1 mg (8.44 mmol) of NEM, 777 mg (4.22 mmol) of thymidin-1-yl-acetic acid and 639 mg (5.06 mmol) of diisopropylcarbodiimide were added to 2.04g (4.22 mmol) of diethyl N-(4-methoxytriphenylmethoxy)ethylamino-methanephosphonate (Example 7), dissolved in 50 ml of absol. DMF. The mixture was stirred at room temperature for 16 h, the solvent was evaporated, the residue was dissolved in DCM and the solution was extracted with saturated aqueous NaHCO₃ solution, then was saturated with aqueous NaCl solution. The extract was dried over sodium sulfate and the solvent evaporated. To purify it, the residue was chromatographed on silica gel (EA/methanol/TEA 98:2:1). The yield was 2.47g (90%).

MS (FAB): 662.3 (M+Na)⁺; 656.3 (M+Li)⁺. ¹H-NMR (200MHz, DMSO, TMS): δ = 1.12-1.32 (m, 6H, CH₂-CH₃); 1.68 & 1.75 (in each case s, 3H, T-CH₃); 3.10-3.40 (m, 2H, CH₂-OMMTr); 3.53-3.70 (m, 4H, P-CH₂ + N-CH₂); 3.75 (s, 3H, OCH₃); 3.83-4.16 (m, 4H, PO-CH₂); 4.62 & 4.72 (in each case s, 2H, CO-CH₂); 6.83-7.42 (m, 15H, Ar-H, T-H); 11.28 (s, 1H, NH).

9) N-Thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonic acid monoethyl ester (tri-ethylammonium salt)

811 mg (1.25 mmol) of diethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 8) were suspended in 3.75 ml of 1N NaOH. The mixture was stirred at room temperature for 3h, then at 50°C

for 6h. The reaction mixture was concentrated in vacuo and the residue was chromatographed on silica gel (EA/methanol/TEA 100:10:10, then 100:40:10). The yield was 897 mg (99.5%).

5

MS (ES-): 620.4 (M-H)⁻. ¹H-NMR (200MHz, DMSO, TMS): δ = 1.18 (t, 9H, N-CH₂-CH₃); 1.68 & 1.74 (in each case s, 3H, T-CH₃); 2.96-3.08 (q, 6H, N-CH₂-CH₃); 3.35 (m, 2H, N-CH₂); 3.43-3.70 (d, J = 11 Hz, 2H, P-CH₂); 3.63 (t, 2H, CH₂-OMMTr); 3.75 (s, 3H, OCH₃); 3.78 (dq, 2H, PO-CH₂); 4.60 & 4.86 (in each case s, 2H, CO-CH₂); 6.82-7.41 (m, 15H, Ar-H, T-H); 11.24 (s, 1H, NH).

10

10) Diethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)ethyl-aminomethanephosphonate

15

The synthesis was carried out analogously to Example 4 from diethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenyl-methoxy)ethylaminomethanephosphonate (Example 8). To purify it, the product was chromatographed on silica gel (EA/methanol 90:10). Yield: 80%.

20

MS (FAB): 400.1 (M+Na)⁺; 378.1 (M+H)⁺. ¹H-NMR (200MHz, DMSO, TMS): δ = 1.17-1.32 (m, 6H, CH₂-CH₃); 1.78 (s, 3H, T-CH₃); 3.40-3.69 (m, 4H, CH₂-OH + N-CH₂); 3.89 (d, J = 11 Hz, 2H, P-CH₂); 3.92-4.19 (m, 4H, PO-CH₂); 4.70 (s, 2H, CO-CH₂); 4.98 (t, 1H, OH); 7.22 & 7.30 (in each case s, 1H, T-H); 11.25 (s, 1H, NH).

25

11) Diphenyl N-(4-methoxytriphenylmethoxy)ethylamino-methanephosphonate

30

The synthesis was carried out analogously to Example 1f, but using diethyl phosphite. Yield: 100%.

35

MS (FAB): 58.2 (M+Li)⁺.

12) N-Thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonic acid monophenyl ester (triethylammonium salt)

5 The synthesis was carried out analogously to Example 81 from diphenyl N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 11) and thymidin-1-yl-acetic acid. To purify it, the product was chromatographed on silica gel (EA/methanol/TEA/H₂O 90:10:5:0.5). Yield: 47%.

10 MS (FAB): 682.3 (M + 2Li-H)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ = 1.16 (t, 9H, N-CH₂-CH₃); 1.67 & 1.72 (in each case s, 3H, T-CH₃); 2.96-3.70 (m, 12H, N-CH₂-CH₃ + N-CH₂ + P-CH₂ + CH₂-OMMTTr); 3.75 (s, 3H, OCH₃); 4.58 & 4.88 (in each case s, 2H, CO-CH₂); 6.74-7.46 (m, 20H, Ar-H, T-H); 11.23 (s, 1H, NH).

15 13) Phenyl 4-nitrophenylethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate

20 385.4mg (0.5 mmol) of N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid monophenyl ester (triethylammonium salt) (Example 12) and 92 mg (0.55 mmol) of 4-nitrophenylethanol were coevaporated three times with absol. pyridine, then dissolved in 15 ml of absol. pyridine. 403.4 mg (0.15 mmol) of 3-nitro-1-(p-toluenesulfonyl)-1H-1,2,4-triazole (TSNT) were added at 0°C, then the mixture was stirred at 0-5°C for 16 h, the pyridine was distilled off in vacuo, the residue was taken up in EA and the solution was washed successively with saturated aqueous NaHCO₃ solution, then with NaCl solution. To purify it, the product was chromatographed on silica gel (EA/TEA 100:2). The yield was 162 mg.

30 MS (FAB): 831.3 (M + 2Li - H)⁺; (M + Li)⁺.

35

14) Di(2-(p-nitrophenyl)ethyl) N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate

The synthesis was carried out analogously to Example 8 from di-(2-(p-nitrophenyl)ethyl) N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 1f) and thymidin-1-yl-acetic acid.

Yield: 63%

MS (ES+): 898.4 (M+Li)⁺. ¹H-NMR (200MHz, DMSO, TMS):

δ = 1.65 & 1.72 (in each case s, 3H, T-CH₃); 2.96 (t, 4H, P-O-CH₂-CH₂-Ar); 3.06 (t, 2H, N-CH₂); 3.67 (d, J = 11Hz, 2H, P-CH₂); 3.70 (m, 2H, MMTr-O-CH₂); 3.75 (s, 3H, OCH₃); 3.83 (s, 3H, OCH₃); 4.10 (dt, 4H, P-O-CH₂); 4.59 & 4.62 (in each case s, broad, 2H, CO-CH₂); 6.83-8.18 (m, 23H, Ar-H, T-H); 11.30 (s, broad, 1H, NH).

15) N-Thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt)

15a) From 30 mg of phenyl 4-nitrophenylethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylamino-methanephosphonate (Example 13)

30 mg of phenyl 4-nitrophenylethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 13) were dissolved in a mixture of 1 ml of TEA, 1 ml of dioxane and 80 mg of p-nitrobenzaloxime and the solution was stirred at room temperature for 3h. The solvent was evaporated in vacuo and the residue was coevaporated three times with pyridine and twice with toluene. The residue was chromatographed on silica gel (EA/TEA 100:2, then EA/methanol/TEA 60:40:2). The yield was 23 mg.

MS (FAB): 755.3 (M + 2Li-H)⁺. ¹H-NMR (200 MHz, DMSO, TMS): δ
 = 1.15 (t, 9H, N-CH₂-CH₃); 1.60 & 1.79 (m, 3H, T-CH₃);
 2.80-3.60 (m, 14H, N-CH₂-CH₃ + N-CH₂ + P-CH₂ + CH₂-OMMTr + Ar-
 CH₂); 3.73 (s, 3H, OCH₃); 4.01 (dt, 2H, P-O-CH₂); 4.58 -4.92
 5 (m, 2H CO-CH₂); 6.82-8.18 (m, 19H, Ar-H, T-H); 11.30 (s, 1H,
 NH).

15b) From di(2-(p-nitrophenyl)ethyl) N-thymin-1-yl-acetyl-N-
 (4-methoxytriphenylmethoxy)ethylamino- methanephosphonate
 10 (Example 14)

The synthesis was carried out analogously to Example 3 from
 di(2-(p-nitrophenyl)ethyl) N-thymin-1-yl-acetyl-N-(4-
 methoxytriphenylmethoxy)ethylaminomethanephosphonate
 15 (Example 14), but in pyridine as a solvent. Yield: 82%.
 Spectroscopic data: see Example 15a.

16) N-Thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-
 ethylaminomethanephosphonic acid
 20

The synthesis was carried out analogously to Example 15b. N-
 Thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethyl-
 aminomethanephosphonic acid is obtained in 18% yield as a
 by-product.
 25

MS (ES⁻): 592.2 (M-H)⁻.

17) 5'-MMTr-T-P(O-ethyl)-T-P-(O-ethyl)₂

30 361 mg (0.5 mmol) of N-thymin-1-yl-acetyl-N-(4-methoxy-
 triphenylmethoxy)ethylaminomethanephosphonic acid monoethyl
 ester (triethylammonium salt) (9) and 188.7 mg (0.5 mmol) of
 diethyl N-thymin-1-yl-acetyl-N-(2-
 35 hydroxy)ethylaminomethanephosphonate (10) were coevaporated
 together twice with absol. pyridine, then dissolved in 10 ml

of absol. pyridine. 1.5 mmol of TSNT were added at 5-10°C and the mixture was stirred at room temperature for 16h. The pyridine was evaporated in vacuo, the residue was dissolved in EA and the solution was washed successively with saturated aqueous NaHCO₃ solution, then NaCl solution. It was dried over Na₂SO₄, concentrated and to purify the residue it was chromatographed on silica gel (EA/methanol/TEA 92:8:2). The yield was 223 mg (46%).

MS (FAB): 987.5 (M+Li)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: Ar-H & thymine-H: 6.82-7.43 (m, 16H); CO-CH₂: 4.59-4.78 (m, 4H); thymine-CH₃: 1.63-1.80 (m, 6H).

18) 5'-HO-T-P(O-ethyl)-T-P(O-ethyl)₂

The synthesis was carried out analogously to Example 4 from 5'-MMTr-T-P(O-ethyl)-T-P(O-ethyl)₂ (Example 17). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 85:15:2, then 100:50:1.5). The yield was 95%.

MS (FAB): 731.2 (M+Na)⁺ 709.1. (M+Li)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: thymine-H: 7.21-7.36 (m, 2H); CO-CH₂: 4.60-4.76 (m, 4H); thymine-CH₃: 1.63-1.79 (m, 6H).

19) 5'-MMTr-T-P(O-phenyl)-T-P(O-ethyl)₂

The synthesis was carried out analogously to Example 17 from diethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)ethyl- amino-methanephosphonate (Example 10) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid monophenyl ester (triethylammonium salt) (Example 12). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 93:7:2). Yield: 58%.

MS (FAB): 1051.4 (M+Na)⁺; 1029.5 (M+H)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: Ar-H & thymine-H: 6.82-7.53 (m, 21H); CO-CH₂: 4.52-4.82 (m, 4H); thymine-CH₃: 1.62-1.80 (m, 6H).

5

20) Di(4-nitrophenylethyl) N-thymin-1-yl-acetyl-N-(2-hydroxyethyl)aminomethanephosponate

10

The synthesis was carried out analogously to Example 4 from di(2-(p-nitrophenyl)ethyl) N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 14). To purify the product, it was chromatographed on silica gel (EA/methanol 90:10). Yield: 85%.

15

MS (ES⁺): 620.3 (M+H)⁺. ¹H-NMR (500MHz, DMSO, TMS): δ = 1.73 (s, 3H, T-CH₃); 2.97 (t, 4H, P-O-CH₂-CH₂-Ar); 3.41 (m, 2H, N-CH₂); 3.59 (m, 2H, CH₂-OH); 3.83 (d, 2H, J = 11Hz; P-CH₂); 4.08-4.30 (m, 4H, P-O-CH₂); 4.54 & 4.78 (in each case s, broad, 2H, CO-CH₂); 4.99 (t, 1H, OH); 7.14-8.19 (m, 9H, Ar-H, thymidiny-H); 11.30 (s, broad, 1H, NH).

20

21) 5'-MMTr-T-P(ONPE)-T-P(O-ethyl)₂

25

The synthesis was carried out analogously to Example 17 from diethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)ethyl-aminomethanephosphonate (Example 10) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylamino-methanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15). Instead of TSNT, 3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1H-1,2,4-triazole (TIPSNT) was employed for the coupling. To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 95:5:2, then 90:10:2). Yield: > 90%.

30

35

MS (ES⁺): 1109.0 (M+Li)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: Ar-H & thymine-H: 6.82-8.18 (m,

20H); CO-CH₂: 4.51-4.76 (m, 4H); thymine-CH₃: 1.61-1.78 (m, 6H).

22) 5'-MMTr-T-P(ONPE)-T-P(OEt)₂,

5

The synthesis was carried out analogously to Example 21 from diethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)ethyl-aminomethanephosphonate (Example 10) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15). Instead of TSNT, 3-nitro-1-(2,4,6-triisopropylphenylsulfonyl)-1H-1,2,4-triazole (TIPSNT) was employed for the coupling. Yield: > 90%.

10

15

For spectroscopic data see Example 21.

23) 5'-HO-T-P(ONPE)-T-P(OEt)₂,

20

Synthesis was carried out analogously to Example 4 from "5'-MMTr-T-P(ONPE)-T-P(OEt)₂" (Example 22). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:2, then 80:20:2). The yield was 75%.

25

MS (ES⁺): 836.3 (M+Li)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: Ar-H & thymine-H: 7.11-8.22 (m, 6H); CO-CH₂: 4.55-4.77 (m, 4H); thymine-CH₃: 1.71 (s, broad, 6H).

30

24). 5'-HO-T-P(OH)-T-P(O-ethyl)₂,

35

10 mg (0.012mmol) of "5'-HO-T-P(ONPE)-T-P(OEt)₂" (Example 23) were dissolved in 1 ml of a 0.5M solution of DBU in pyridine and stirred first at 4°C for 24 h, then at room temperature for 24 h. The solvent was evaporated in vacuo, and the residue was digested twice with pentane, then twice

with ether, then to purify the product it was chromatographed on silica gel (EA/methanol/TEA 9:1:0.2, then 70:30:2, then 60:40:2). Yield: 10.2 mg.

5 MS (FAB): 725.3 (M + 2Na-H)⁺; 703.3 (M+Na)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: thymine-H: 7.15-7.70 (m, 2H); CO-CH₂: 4.67-4.92 (m, 4H); thymine-CH₃: 1.67-1.81 (m, 6H).

10 25) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

The synthesis was carried out analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(OEt)₂" (Example 22) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-

15 ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15) with addition of 1.5 eq (based on Example 23) of 4-methoxypyridine-N-oxide. To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:2, then 85:15:2). Yield: 20 61%.

MS (ES⁺): 1555.8 (M+H)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: Ar-H & thymine-H: 6.83-8.20 (m, 25 25H); CO-CH₂: 4.52-4.75 (m, 6H); thymine-CH₃: 1.61-1.78 (m, 9H).

26) 5'-HO-T-P-(ONPE)-T-P(ONPE)-T-P(OEt)₂

The synthesis was carried out analogously to Example 4 from 30 "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 25). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 70:30:2). The yield was 89%.

MS (ES⁺): 1283.1 (M+H)⁺; 1305.0 (M+Na⁺).

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27) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

The synthesis was carried out analogously to Example 17 from "5'-HO-T-P-(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 26) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15) with addition of 1.5 eq (based on Example 23) of 4-methoxypyridine-N-oxide. To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:2, then 80:20:2). Yield: 15%.

MS (ES⁺): 2007 (M+H)⁺; 2029 (M+Na)⁺. ¹H-NMR (200MHz, DMSO, TMS) Characteristic signals are: Ar-H & thymine-H: 6.79-8.21 (m, 30H); CO-CH₂: 4.53-4.87 (m, 8H); thymine-CH₃: 1.58-1.89 (m, 12H).

28) 5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

The synthesis was carried out analogously to Example 4 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 27). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 70:30:2). The yield was 55%.

MS (FAB): 1735 (M+H)⁺; 1757 (M+Na)⁺.

29) 5'-MMTr-T-P(ONPE)-T-P(ONPE)₂

The synthesis was carried out analogously to Example 17 from di(4-nitrophenylethyl) N-thymin-1-yl-acetyl-N-(2-hydroxyethyl)aminomethanephosphonate (Example 20) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 100:10:1, then 90:10:1). Yield: 87%.

MS (FAB): 1356.2 (M + 2Li-H)⁺. ¹H-NMR (200MHz, DMSO, TMS)
 Characteristic signals are: Ar-H & thymine-H: 6.82-8.18 (m, 28H); CO-CH₂: 4.50-4.71 (m, 4H); thymine-CH₃: 1.59-1.78 (m, 6H).

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30) 5'-HO-T-P(ONPE)-T-P(ONPE)₂

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The synthesis was carried out analogously to Example 4 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)₂" (Example 29). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 85:15:1, then 80:20:1). The yield was 78%.

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MS (ES⁺): 1072.7 (M+H)⁺. ¹H-NMR (200MHz, DMSO, TMS)
 Characteristic signals are: Ar-H & thymine-H: 7.08-8.20 (m, 14H); CO-CH₂: 4.52-4.80 (m, 4H); thymine-CH₃: 1.70 (s, broad, 6H).

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31) 5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂

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The synthesis was carried out analogously to Example 17 from N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytri-phenylmethoxy)ethylaminomethanephosphonic acid 2-(p-nitrophenyl)ethyl monoester (triethylammonium salt) (Example 3) and "5'-HO-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂" (Example 6). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:19:1). Yield: 66%.

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MS (FAB): 2155 (M+H)⁺; 2161 (M+Li)⁺; 2177 (M+Na)⁺.

32) 5'-HO-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂

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The synthesis was carried out analogously to Example 4 from "5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂" (Example 31). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 85:15:1, then 80:20:1). The yield was 70%.

MS (FAB): 1882 (M+H)⁺; 1904 (M+Na)⁺.

33) Allyl 2-(p-nitrophenyl)ethyl N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethane-phosphonate

The synthesis was carried out analogously to Example 17 from N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 2-(p-nitrophenyl)ethyl monoester (triethylammonium salt) (Example 3) and allyl alcohol. To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 95:5:1).

MS (ES⁺): 902.1 (M+H)⁺; 924.1 (M+Na)⁺. ¹H-NMR (200MHz, DMSO, TMS): δ = 2.94-3.70 (m, 8H, P-O-CH₂-CH₂-Ar + MMTr-O-CH₂ + N-CH₂ + P-CH₂); 3.75 (s, 3H, OCH₃); 3.86 (s, 3H, OCH₃); 4.10-4.60 (m, 4H, P-O-CH₂); 4.79 & 4.84 (in each case s, broad, 2H, CO-CH₂); 5.09-5.39 (m, 2H, H₂C = CH-); 5.71-6.00 (m, 1H, H₂C=CH-); 6.83-8.19 (m, 24H, Ar-H, cytosinyl-H); 11.03 (s, broad, 1H, NH).

34) Allyl 2-(p-nitrophenyl)ethyl N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(2-hydroxy)ethylaminomethanephosphonate

The synthesis was carried out analogously to Example 4 from allyl 2-(p-nitrophenyl)ethyl N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 33). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 94:5:1). The yield was 83%.

MS (ES⁺): 630.2 (M+H)⁺. ¹H-NMR (200MHz, DMSO, TMS): δ = 3.02 (t, 2H, P-O-CH₂-CH₂-Ar); 3.37-3.72 (m, 4H, HO-CH₂-CH₂); 3.86 (s, 3H, OCH₃); 3.91 (d, J = 11 Hz, 2H, P-CH₂); 4.22 (dt, 2H, P-O-CH₂-CH₂-Ar); 4.40 (dd, 2H, O-CH₂-CH=CH₂); 4.78 & 5.01 (m, 2H, CO-CH₂); 5.11-5.33 (m, 2H, H₂C = CH-); 5.71-6.00 (m, 1H,

$H_2C = \underline{CH}$ -); 6.99-8.21 (m, 14H, Ar-H, cytosinyl-H); 11.03 (s, broad, 1H, NH).

35) Allyl 2-(p-nitrophenyl)ethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate

The synthesis was carried out analogously to Example 17 from N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15) and allyl alcohol. To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 97:3:2). The yield was 100%.

MS (FAB): 805.3 (M+Na)⁺.

36) Allyl 2-(p-nitrophenyl)ethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)ethylaminomethanephosphonate

Synthesis analogously to Example 4 from allyl 2-(p-nitrophenyl)ethyl N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 35). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:2). The yield was 86%.

MS (ES⁺): 511.1 (M+H)⁺

37) 5'-MMTr-T-P(ONPE)-T-P(ONPE)(O-allyl)

Synthesis analogously to Example 17 from allyl 2-(p-nitrophenyl)ethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)-ethylaminomethanephosphonate (Example 36) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:2). Yield: 90%.

MS (FAB): 1257.3 (M+Na)⁺.

38) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

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Synthesis analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 28) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethyl-aminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:20:2). Yield: 57%.

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MS (FAB): 2460 (M+H)⁺; 2482 (M+Na)⁺.

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39) 5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 4 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 38). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 70:30:2). The yield was 55%.

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MS (FAB): 2209 (M+Na)⁺.

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40) 5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OEt)₂

4.0 mg (0.00183 mmol) of "5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 39) were dissolved in 1.1 ml of a 0.5 molar solution of DBU in pyridine and stirred at room temperature for 24 h. The reaction mixture was evaporated in vacuo and the residue was stirred several times with toluene. The solvent was removed using a syringe, the residue was stirred again with pentane, and the solvent was removed again using a syringe. The product was dried in vacuo. The yield was 4mg of a strongly hygroscopic powder.

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MS (ES-): 1589.7 (M-H)⁻; 1611.8 (M+Na-2H)⁻.

41) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

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a) Synthesis analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 39) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)-ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:20:2, then 70:30:2).

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MS (FAB): 2934 (M+Na)⁺, 2957 (M + 2Na-H)⁺, 2978 (M + 3Na-2H)⁺.

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b) Synthesis analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 28) and "5'-MMTr-T-P(ONPE)-T-P(ONPE)(OH)" (Example 42). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:20:2, then 70:30:2). The target fraction was evaporated in vacuo, then triturated with pentane and ether. MS as above.

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42) 5'-MMTr-T-P(ONPE)-T-P(ONPE)(OH)

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24.7 mg (0.02 mmol) of "5'-MMTr-T-P(ONPE)-T-P(ONPE)(O-allyl)" (Example 37) were dissolved in 2 ml of absol. DCM together with 16.2 mg (0.12 mmol) of diethylammonium hydrogencarbonate. At 15-20°C, a solution of 13.9 mg (0.012 mmol) of tetrakis(triphenylphosphine)palladium (0) and 2.1 mg (0.008 mmol) of triphenylphosphine in 2 ml of absol. DCM was added dropwise during the course of 2 min. The mixture was stirred at room temp. for 30 min. To purify the product, the reaction mixture was chromatographed on silica gel (EA/methanol/TEA 80:20:1, then 60:40:1). The product

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fraction was evaporated in vacuo, and the residue was triturated with pentane, then with EA/ether, then again with pentane and dried in vacuo.

Yield: 57%.

MS (ES⁻): 1193.6 (M-H)⁻.

¹H-NMR (200MHz, DMSO, TMS): characteristic signals: δ = 1.67 & 1.72 (in each case s, 3H, T-CH₃); 4.60 & 4.82 (in each case s, 2H, CO-CH₂); 6.83-8.19 (m, 24H, Ar-H, T-H);

43) 5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 4 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 41). After reaction had taken place, the reaction mixture was concentrated, and the residue was coevaporated three times with toluene, then stirred, first with EA/ether, then with pentane. The residue was dried in vacuo.

MS (FAB): 2662 (M+Na)⁺.

44) 5'-HO-T-P(ONPE)-T-P(ONPE)(O-allyl)

Synthesis analogously to Example 4 from 5'-MMTr-T-P(ONPE)-T-P(ONPE)(O-allyl) (Example 37). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:1, then 80:20:1). The yield was 87%.

MS (FAB): 963.0 (M+H)⁺; 985.1 (M+Na)⁺.

45) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(OH)

a) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(O-allyl)

Synthesis analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(ONPE)(O-allyl)" (Example 44) and N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:1, then 85:15:1). Yield: 55%

b) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(OH)

"5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(O-allyl)" (Example 45a) was reacted with tetrakis(triphenylphosphine)palladium (0) as described in Example 42. To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:20:2, then 70:30:2). The product fraction was evaporated in vacuo, and the residue was triturated with pentane and ether. Yield: 98%.

MS (ES⁺; LiCl): 1654.1 (M+Li⁺)

46) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 17 from diethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)ethylaminomethane-phosphonate (Example 10) and "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(OH)" (Example 45b). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 90:10:2, then 80:20:2). Working up, purification and characterization as in Example 27.

47) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 26) and "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(OH)" (Example 45b). To purify the product,

it was chromatographed on silica gel (EA/methanol/TEA 80:20:2). The product fraction was evaporated in vacuo, coevaporated with toluene and purified by preparative HPLC (high-pressure liquid chromatography): RP8 LiChrospher 60, water/acetonitrile/ : 1:1; 0.1% ammonium acetate; 1 ml/min. R_f = 12.97 min.

48) 5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OEt)₂

a) 5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 4 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 47). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 70:30:2, then 60:40:2). The product fraction was evaporated in vacuo, and the residue was stirred, first with pentane, then with ether, and dried in vacuo. Yield: 100%.

MS (FAB): 2662 (M+Na)⁺, 2684 (M + 2Na-H)⁺, 2706 (M + 3Na-2H)⁺.

b) 5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OEt)₂

"5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 48a) was reacted with DBU and worked up analogously to Example 40.

MS (ES⁻): 1892 (M-H⁻); 1915 (M+Na-2H)⁻.

49) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 28) and "5'-MMTr-T-

P(ONPE)-T-P(ONPE)-T-P(ONPE)(OH)" (Example 45b). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:20:2, then 70:30:2). The product fraction was evaporated in vacuo, coevaporated with toluene and purified by preparative HPLC: RP8 LiChrospher 60, water/acetonitrile: 1:1; 0.1% ammonium acetate; 1 ml/min. R_f = 15.24 min.

MS (FAB): 3386 (M+Na)⁺, 3409 (M + 2Na-H)⁺,

50) 5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 4 from "5'MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 49). To purify the product, it was evaporated in vacuo and coevaporated three times with toluene, and the residue was stirred, first with pentane, then with ether, and dried in vacuo. Yield: > 90%.

MS (FAB): 3114 (M+Na)⁺

51) 5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OEt)₂

"5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 50) was reacted with DBU and worked up analogously to Example 40.

MS (ES-): 2196 (M-H)⁻; 2218 (M + Na-2H)⁻.

52) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 17 from "5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example

48a) and "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(OH)" (Example 45b). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 70:30:2, then 60:40:2). The product fraction was evaporated in vacuo, coevaporated with toluene and purified by preparative HPLC: RP8 LiChrospher 60, water/acetonitrile: 1:1; 0.1% ammonium acetate; 1 ml/min. R_f = 23.95 min.

53) 5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OEt)₂

a) 5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂

Synthesis analogously to Example 4 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 52). To purify the product, it was evaporated in vacuo and coevaporated three times with toluene, and the residue was stirred, first with pentane, then with ether, and dried in vacuo. Yield: > 90%.

b) 5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OEt)₂

"5'-HO-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 53a) was reacted with DBU and worked up analogously to Example 40.

MS (ES⁻): 2802 (M-H⁻); 2825 (M + Na-2H⁻).

54) Di(2-(p-nitrophenyl)ethyl) 2-(N'-tert-butoxycarbonylamino)ethylaminomethanephosphate

a) 1-Methylimino-2-(N'-tert-butoxycarbonylamino)ethane (trimer)

2.0 g (12.5 mmol) of 2-amino-1-(N'-tert-butoxycarbonylamino)ethane, dissolved in 8 ml of methanol, were treated with ice cooling with 1.52 ml (18.72 mmol) of 37% formaldehyde and the mixture was stirred at room temp. for 1 h. The residue was taken up in EA, washed twice with saturated NaHCO₃ solution, then with NaCl solution, dried, filtered and evaporated in vacuo. To purify the product, it was chromatographed on silica gel (EA/TEA 100:0.2, then EA/methanol/TEA 90:10:0.2). The yield was 0.8 g.

MS (FAB/LiCl) 523.4 (M + 2Li-H)⁺. ¹H-NMR (200MHz, DMSO, TMS): δ = 1.38 (s, 27H, tBu-H); 2.40 (t, 6H, N-CH₂); 2.99 (t, 6H, N-CH₂); 3.25 (t, 6H, N-CH₂); 6.81 (t, broad, 3H, NH).

b) 2-Di(2-(p-nitrophenyl)ethyl) 2-(N'-tert-butoxycarbonylamino)ethylaminomethanephosphonate

1-Methylimino-2-(N'-tert-butoxycarbonylamino)ethane (trimer) (Example 54a) was reacted with di(2-(4-nitrophenyl)ethyl) phosphite (Example 1e) analogously to Example 1f. To purify the product, it was chromatographed on silica gel (first toluene/EA/TEA 20:80:0.2; then EA/TEA 100:0.2, then EA/methanol/TEA 0.5:5:0.2). Yield: 25%.

MS(ES⁺/LiCl) 553.3 (M+H)⁺, 559.3 M+Li)⁺, ¹H-NMR (200MHz, DMSO, TMS): δ = 1.37 (s, 9H, tBu-H); 2.83 (d, J=12Hz, 2H, P-CH₂); 2.55 (t, 4H, Ar-CH₂); 2.90-3.09 (m, 4H, N-CH₂-CH₂-N); 4.16 (dt, 4H, PO-CH₂); 7.52 & 8.15 (in each case d, 8H, Ar-H).

55) Di-(2-(p-nitrophenyl)ethyl N-thymin-1-yl-acetyl-N-(2-N'-tert-butoxycarbonylamino)ethylaminomethanephosphonate

Synthesis analogously to Example 8 from di(2-(p-nitrophenyl)ethyl) 2-(N'-tert-butoxycarbonylamino)ethylamino-m-

ethanephosphonate (Example 54b) and thymidin-1-yl-acetic acid.

Yield: 86%

MS (FAB/LiCl): 725.3 (M+Li)⁺. ¹H-NMR (200MHz, DMSO, TMS): δ = 1.42 (s, 9H, tBu-H); 1.91 (s, 3H, T-CH₃); 2.99-3.58 (m, 8H, P-O-CH₂-CH₂-Ar & N-CH₂-CH₂-N); 3.75 (d, J=12Hz, 2H, P-CH₂); 4.06-4.38 (m, 4H, PO-CH₂); 7.37 & 8.15 (in each case d, 8H, Ar-H).

56) Interaction with DNA: UV melting curve

The interaction of the compounds according to the invention with complementary nucleic acids was demonstrated by way of example by the recording of the UV melting curve of "5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P(OEt)₂" (Example 53b) with (dA)_n. To do this, a 0.3 OD of "5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P(OEt)₂" and (dA)_n, in each case was prepared in 1 ml of a buffer (1 M NaCl, 20 mM MgCl₂, 10 mM HEPES, pH 7.5) and the change in extinction at 260 nm was recorded as a function of the temperature (0 to 80°C). The results can be seen in FIG 1. A T_m value of about 23°C was determined from the melting curve obtained.

57) Interaction with DNA: gel shift experiment

The interaction of the compounds according to the invention with complementary nucleic acids was demonstrated by way of example by the hybridization of "5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P(OEt)₂" (Example 53b) with (dA)_n, in a gel shift experiment. To do this, "5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P(OEt)₂" (Example 53b) and (dA)_n, were in each case applied on their own and mixed in the ratio 1:1, 1:2, 1:5 and 1:10 to a nondenatured

polyacrylamide gel (20%, running buffer 1 x TBE, 10mM MgCl₂) and the running behavior determined. The results can be seen in FIG. 2: (dA), runs faster than "5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P(OH)-T-P(OEt)₂," and in the 1:1 mixture both can only be seen weakly, instead a slower band is formed which corresponds to a complex between the two components. In the 2:1 mixture, (dA), can no longer be seen, instead the new band is all the more distinct. The same applies for the 5:1 or 10:1 mixture, in which additionally the distinct excess of "5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P-(OH)-T-P(OH)-T-P(OEt)₂," can be detected.

58) 5'-MMTr-T-P(ONPE)-T-P(Oethyl)₂, [see also Example 21, alternative syntheses]

a) 8.44 mg (10 μmol) of N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt) (Example 15), 3.77 mg (10 μmol) of diethyl N-thymin-1-yl-acetyl-N-(2-hydroxy)ethylaminomethanephosphonate (Example 10) and 64.6 mg (500 μmol) of N-ethyldiisopropylamine (DIPEA) were dissolved in 0.3 ml of absol. DMF. 44.2 mg (100 μmol) of benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) were added thereto. The mixture was stirred at room temp. for 24h. About 70% yield according to TLC (EA/methanol/TEA 100:20:2; R_f = 0.5).

b) Analogously to Example 58a, but using 30 μmol of HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate) instead of 100 μmol of BOP. About 65% yield according to TLC.

59) 2-(p-Nitrophenyl)ethyl 5'-(3'-levuloyl) thymidine N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate

Synthesis according to Example 17 from N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 4-nitrophenylethyl monoester (triethylammonium salt, Example 15) and 3'-levuloylthymidine. To purify the product, it was chromatographed on silica gel (DCM/methanol/TEA 98:2:0.25). The yield was 46%.

MS (FAB/LiCl): 1071.4 (M+Li)⁺.

60) N-Thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 2-(p-nitrophenyl)ethyl 5'-thymidine diester

64 mg (0.06 mmol) of 2-(p-nitrophenyl)ethyl 5'-(3'-levuloyl thymidine N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 59) were dissolved in 0.5 ml of dioxane. 9 mg (0.23 mmol) of NaBH₄ in 0.12 ml of water were added and the mixture was stirred at room temperature for 20 min. The solvent was evaporated in vacuo, the residue was taken up in DCM, and the solution was extracted with water and dried. To purify the product, it was chromatographed on silica gel (DCM/methanol/TEA 92:8:0.5). The yield was 72%.

MS (FAB/LiCl): 973.4 (M+Li)⁺, 979.4 (M + 2Li-H)⁺, 985.4 (M + 3Li-2H)⁺.

61) N-Thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 2-(p-(nitrophenyl)ethyl 5'-thymidin-3'-(cyanoethyl-N,N-diisopropyl phosphoramidite)

31 mg (0.032 mmol) of N-thymin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid 2-(p-nitrophenyl)ethyl 5'-thymidine diester (Example 60) were coevaporated twice with absol. CH₃CN and dissolved in 0.4 ml absol. THF. First 12.4 mg (0.096 mmol) of

diisopropylethylamine, then 9.8 mg (0.045 mmol) of cyanoethyl chlorodiisopropylphosphoramidite were added thereto. The mixture was stirred for 3h, filtered and evaporated in vacuo. The yield was 63%.

MS (FAB/LiCl): 1173.3 (M+Li)⁺, 1180.4 (M + 2Li-H)⁺, 1186.4 (M + 3Li-2H)⁺.

62) Di(2-(p-nitrophenyl)ethyl) N-[N9-(O6-diphenylcarbamoyl-N2-acetylguanine]acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosponate

Synthesis analogously to Example 2 from di(2-(p-nitrophenyl)ethyl) N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 1f) and O6-diphenylcarbamoyl-N2-acetylguanineacetic acid. To purify the product, it was chromatographed on silica gel (EA/TEA 98:2). Yield: 87%

MS (FAB/LiCl): 1154.8 (M+H)⁺, 1160.7 (M+Li)⁺.

63) Di(2-(p-nitrophenyl)ethyl, N-[N9-(N4-anisoyladenine]-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosponate

Synthesis analogously to Example 2 from di(2-(p-nitrophenyl)ethyl, N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 1f) and N4-anisoyladenineacetic acid. To purify the product, it was chromatographed on silica gel (DCM/methanol/TEA 95:4:1). Yield: 82%; MS (ES⁺): 1035.7 (M+H)⁺. ¹H-NMR (200MHz, DMSO, TMS): δ = 2.93 (t, 4H, P-O-CH₂-CH₂-Ar); 3.09 (t, 2H, MMTr-O-CH₂); 3.23-3.75 (m, 4H, P-CH₂ + N-CH₂); 3.75 (s, 3H, OCH₃); 3.87 (s, 3H, OCH₃); 4.08 (dt, 4H, P-O-CH₂); 5.28-5.42 (m, 2H, CO-CH₂); 6.81-8.20 (m, 28H, Ar-H, A-H); 11.00 (s broad, 1H, NH).

64) N-[N9-(N4-Anisoyladenine)acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid mono(2-(p-nitrophenyl)ethyl) ester

5 Synthesis analogously to Example 3 from di(2-(p-nitrophenyl)ethyl) N-[N9-(N4-anisoyladenine)acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonate (Example 63). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 65:35:2). The yield was 52%.

10 MS (FAB/LiCl): 874.3 (M + 2Li-H)⁺.

65) Di(2-(p-nitrophenyl)ethyl), N-thymin-1-yl-acetyl-N-(2-methoxy)ethylaminomethanephosphonate

15 Synthesis analogously to Example 2 from di(2-(p-nitrophenyl)ethyl) N-(2-methoxy)ethylaminomethanephosphonate (prepared analogously to Example 1 starting from 2-methoxyethylamine by reaction with formaldehyde and di(2-(4-nitrophenyl)ethyl phosphite) and thymidin-1-yl-acetic acid. To purify the product, it was chromatographed on silica gel (EA/methanol 90:10). Yield: 64%

20 MS (FAB/LiCl): 640.3 (M+Li)⁺.

25 66) 5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)(OAllyl)

30 Synthesis analogously to Example 17 from N-(N⁶-anisoyl)-cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethylaminomethanephosphonic acid (2-(p-nitrophenyl)ethyl) monoester (triethylammonium salt) (Example 3) and allyl 2-(p-nitrophenyl)ethyl N-(N⁶-anisoyl)cytosin-1-yl-acetyl-N-(2-hydroxy)ethylaminomethanephosphonate (Example 34). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 85:14:1). Yield: 36%.

MS (FAB): 1474 (M+H)⁺; 1496 (M+Na)⁺.

67) 5'-HO-C^{An}-P(ONPE)-C^{An}-P(ONPE)(Oallyl)

5 Synthesis analogously to Example 4 from "5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)(Oallyl)" (Example 66). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:19:1). The yield was 55%.

10 MS (FAB): 1201.3 (M+H)⁺, 1223.3 (M+Na)⁺.

68) 5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)(O-allyl)

15 Synthesis analogously to Example 17 from N-(N⁶-anisoyl)-cytosin-1-yl-acetyl-N-(4-methoxytriphenylmethoxy)ethyl-aminomethanephosphonic acid 2-(p-nitrophenyl)ethyl monoester (triethylammonium salt) (Example 3) and 5'-HO-C^{An}-P(ONPE)-C^{An}-P(ONPE)(Oallyl) (Example 67). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 80:20:1).
20 Yield 58%.

MS (FAB): 2046 (M+H)⁺; 2068 (M+Na)⁺.

69) 5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)(OH)

25 Synthesis analogously to Example 42 from 5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)(O-allyl) (Example 68). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 60:38:2). Yield: 66%.

30 MS (FAB): 2027 (M+Na)⁺; 2049 (M + 2Na-H)⁺.

70) 5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂

35

Synthesis analogously to Example 17 from "5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)(OH)" (Example 69) and "5'-HO-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂" (Example 32). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 70:30:2). Yield: 58%.

MS (FAB): 3892 (M+Na)⁺; 3914 (M + 2Na-H)⁺.

71) 5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂

Synthesis analogously to Example 17 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)(OH)" (Example 45) and "5'-HO-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂" (Example 32). To purify the product, it was chromatographed on silica gel (EA/methanol/TEA 60:40:2). The product fraction was evaporated in vacuo and purified by preparative HPLC (high pressure liquid chromatography): RP8 LiChrospher 60, water/acetonitrile: 1:1; 0.1% ammonium acetate; 1ml/min) R_f = 16.6 min

MS (FAB): 3534 (M+Na)⁺; 3556 (M+2Na-H)⁺.

72) 5'-MMTr-T-P(OH)-T-P(OH)-T-P(OH)-C^{An}-P(OH)-C^{An}-P(OH)-C^{An}-P(OH)₂

Synthesis analogously to Example 40 from "5'-MMTr-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)₂" (2 mg) (Example 71).

MS (ES-): 2466.4 (M-H).

73) 5'-MMTr-T-P(OH)-T-P(OH)-T-P(OH)-C-P(OH)-C-P(OH)-C-P(OH)₂

About 1 mg of "5'-MMTr-T-P(OH)-T-P(OH)-T-P(OH)-C^{An}-P(OH)-C^{An}-P(OH)-C^{An}-P(OH)₂" (Example 72) was treated with 3 ml of 33%

aqueous NH_4OH and stirred at room temperature for 24 h. The reaction mixture was evaporated in vacuo. Yield: about 0.6 mg (19 OD)

5 MS (ES-): 2064.5 (M-H)⁻.

74) 5'-HO-T-P(OH)-T-P(OH)-T-P(OH)-C-P(OH)-C-P(OH)-C-P(OH)₂

10 8 OD of "5'-MMTr-T-P(OH)-T-P(OH)-T-P(OH)-C-P(OH)-C-P(OH)-C-P(OH)₂" (Example 73) were dissolved in 0.5 ml of water and added to a PolyPak™ (Glen Research, #60-1100-10). The MMTr group was removed following the instructions of the manufacturer (Glen Research User Guide). Yield: about 0.35 mg (11 OD).

15 MS (ES-): 1792.6 (M-H)⁻.

75) 5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)-T-P(ONPE)-T-P(ONPE)-T-P(ONPE)₂

20 Synthesis analogously to Example 17 from "5'-MMTr-C^{An}-P(ONPE)-C^{An}-P(ONPE)-C^{An}-P(ONPE)(OH)" (Example 69) and "5'-HO-T-P-(ONPE)-T-P(ONPE)-T-P(OEt)₂" (Example 26). To purify the product, it was chromatographed on silica gel
25 (EA/methanol/TEA 80:20:2, then 70:30:2). The product fraction was coevaporated with toluene, evaporated in vacuo and triturated with pentane. Yield: 48%.

MS (FAB): 3744 (M+Na)⁺; 3766 (M + 2Na-H)⁺.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (iii) APPLICANT:
(A) NAME: Hoechst Aktiengesellschaft
(B) STREET: -
(C) CITY: Frankfurt
(D) FEDERAL STATE: -
(E) COUNTRY: Germany
(F) POSTAL CODE: 65926
(G) TELEPHONE: -
(H) TELEFAX: -
(I) TELEX: -
- (ii) TITLE OF APPLICATION: Phosphonomonoester nucleic acids, process for their preparation, and their use
- (iii) NUMBER OF SEQUENCES: 33
- (iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0. Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

- (ix) FEATURES:
(A) NAME/KEY: exon
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ACACCCAATT CTGAAAATGG

20

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

- (ix) FEATURES:
(A) NAME/KEY: exon
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
AGGTCCTGT TCGGGCGCCA

20

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

- (ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
GTCGACACCC AATTCTGAAA ATGGATAA

28

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

- (ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
GCTATGTCGA CACCCAATTC TGA

25

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

- (ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..26

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GTGGCTGTCT CCGCTTCTTC TTCCTG

26

(2) INFORMATION FOR SEQ ID NO: 6:

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..27

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GTCTCCGCTT CTTCCTCCTG CCATAAG

27

(2) INFORMATION FOR SEQ ID NO: 7:

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..20

(x1) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GCGGGGCTCC ATGGGGGTCC

20

(2) INFORMATION FOR SEQ ID NO: 8:

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
CAGCTGCAAC CCAGC

15

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

- (ix) FEATURES:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
GGCTGCTGGA GCGGGGCACA C

21

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

- (ix) FEATURES:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
AACOTTGAGG GGCAT

15

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTGCCCGGGT CTTGGGC

18

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGAGAACATC ATGTCGAAA G

21

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCCGAGAACA TCATGGTCGA AG

22

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGGGAAAGCC CGGCAAGGGG

20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CACCCGCCTT GGCCTCCAC

20

(2) INFORMATION FOR SEQ ID NO: 16

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNS (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GGGACTCCGG CGCAGCGC

18

(2) INFORMATION FOR SEQ ID NO: 17

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGCAAACCTT CTTTTCCTCC

20

(2) INFORMATION FOR SEQ ID NO: 18

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGGAAGGAGG AGGATGAGG

19

(2) INFORMATION FOR SEQ ID NO: 19

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

- (A) NAME/KEY: exon
- (B) LOCATION: 1..21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GGCAGTCATC CAGCTTCGGA G

21

(2) INFORMATION FOR SEQ ID NO: 20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii) ANTISENSE: yes
(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..18
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:
GCAGTAAGCA TCCATATC 18

(2) INFORMATION FOR SEQ ID NO: 21
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) ANTISENSE: yes
(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..20
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
CCCCCACCAC TTCCCTCTC 20

(2) INFORMATION FOR SEQ ID NO: 22
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) ANTISENSE: yes
(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..20
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:
CTCCCCCACC ACTTCCCCTC 20

(2) INFORMATION FOR SEQ ID NO: 23
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) ANTISENSE: yes
(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..19
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:
GCTGGGAGCC ATAGCGAGG 19

(2) INFORMATION FOR SEQ ID NO: 24
(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) ANTISENSE: yes
(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..21
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:
ACTGCTGCCT CTTGTCTCAG G 21

(2) INFORMATION FOR SEQ ID NO: 25
(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) ANTISENSE: yes
(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..22
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:
CAATCAATGA CTTCAAGAGT TC 22

(2) INFORMATION FOR SEQ ID NO: 26
(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

(A) NAME/KEY: exon
(B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GGTCCCTGTT CGGGCGCCA

19

(2) INFORMATION FOR SEQ ID NO: 27

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

(A) NAME/KEY: exon
(B) LOCATION: 1..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GTGCCGGGGT CTTGGG

17

(2) INFORMATION FOR SEQ ID NO: 28

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTISENSE: yes

(ix) FEATURES:

(A) NAME/KEY: exon
(B) LOCATION: 1..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GGAGGATGCT GAGGAGG

17

(2) INFORMATION FOR SEQ ID NO: 29

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GGAGGATGCT GAGG 14

(2) INFORMATION FOR SEQ ID NO: 30

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CAGGAGGATG CTGAGGAGG 19

(2) INFORMATION FOR SEQ ID NO: 31

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) ANTISENSE: yes

(ix) FEATURES:
 (A) NAME/KEY: exon
 (B) LOCATION: 1..15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GGCTGCCATG GTCCC 15

(2) INFORMATION FOR SEQ ID N : 32

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTISENSE: yes
- (ix) FEATURES:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1..20
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

TCATGGTGTC CTTGCAGCC

20

(2) INFORMATION FOR SEQ ID NO: 33

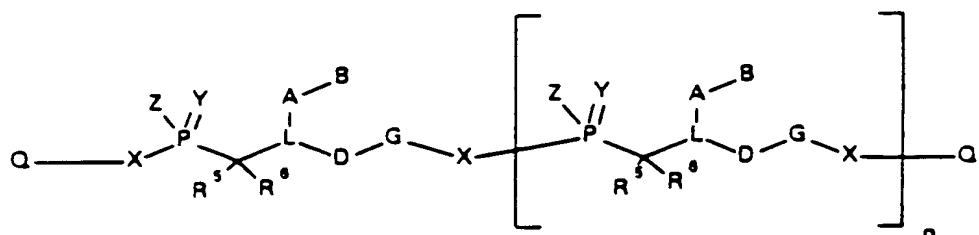
- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTISENSE: yes
- (ix) FEATURES:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 1..18
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TCATGGTGTC CTTGCAG

18

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A compound of formula I



(I)

in which n is a number from zero to 100;

B independently of one another is hydrogen, hydroxyl, (C₁-C₂₀)-alkyl, (C₁-C₂₀)-alkoxy, (C₁-C₂₀)-alkylthio, (C₆-C₂₀)-aryl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkyl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkoxy, (C₆-C₂₀)-aryl-(C₁-C₆)-alkylthio, an aromatic group or a heterocyclic group, wherein alkyl, aryl, aromatic or heterocyclic group is optionally substituted one or more times by hydroxyl, (C₁-C₄)-alkoxy, -NR⁹R¹⁰, -C(O)OH, oxo, -C(O)OR⁸, -C(O)NR⁹R¹⁰, -CN, -F, -Cl, -Br, -NO₂, (C₂-C₆)-alkoxyalkyl, -S(O)_nR⁸, -(C₁-C₆)-alkyl-S(O)_nR⁸, -NHC(=NH)NHR⁸, -C(=NH)NHR⁸, -NR⁹C(=O)R⁸, =NOR⁸, NR⁹C(=O)OR¹⁰, -OC(=O)NR⁹R¹⁰ or -NR⁹C(=O)NR⁹R¹⁰,

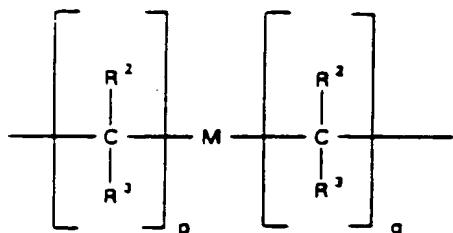
B independently of one another is a natural nucleobase, an unnatural nucleobase or a reporter ligand;

A-B is a D- or L-amino acid condensed on via the carboxyl group or peptides consisting of amino acids having a length of up to 5 amino acid residues,

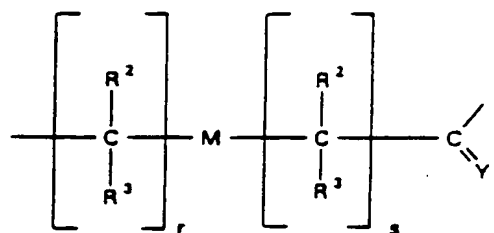
L independently of one another is N or R¹N^{*}, and

R^1 is hydrogen or (C_1-C_6) -alkyl optionally substituted by hydroxyl, (C_1-C_6) -alkoxy, (C_1-C_6) -alkylthio or amino;

A independently of one another is a single bond, a methylene group or a group of formula IIa or IIb;



(IIa)



(IIb)

Y' is $=O$, $=S$, $=CH_2$, $=C(CH_3)_2$ or $=NR^1$, where R^1 is as defined above;

M is a single bond, $-O-$, $-S-$ or $-NR^1-$, where R^1 is as defined above;

R^2 and R^3 independently of one another are hydrogen, hydroxyl, (C_1-C_6) -alkoxy, (C_1-C_6) -alkylthio, amino, halogen, or (C_1-C_6) -alkyl optionally substituted by hydroxyl, (C_1-C_6) -alkoxy or (C_1-C_6) -alkylthio;

p and q independently of one another are zero to 5;

r and s independently of one another are zero to 5;

D and G each independently represent CR^5R^6 which can be the same or different;

R^5 and R^6 independently of one another are hydrogen, (C_1-C_6) -alkyl, (C_6-C_{20}) -aryl, (C_6-C_{20}) -aryl- (C_1-C_6) -alkyl, hydroxyl, (C_1-C_6) -alkoxy, (C_1-C_6) -alkylthio, wherein alkyl and aryl are optionally substituted by SR^1 or

$\text{NR}^1\text{R}^{1'}$, where R^1 is as defined above and $\text{R}^{1'}$ independently of R^1 has the same meaning as R^1 ;

X is -O-, -S- or -NR¹-, in which R^1 is as defined above;

Y is =O or =S;

Z is -OR⁸, -NR⁹R¹⁰ or X'Q", where X' is defined as X and Q" is defined as Q;

R⁸ is hydrogen, (C₁-C₁₈)-alkyl, (C₂-C₁₈)-alkenyl, (C₃-C₁₈)-alkynyl, (C₆-C₁₂)-aryl, (C₆-C₁₂)-aryl-(C₁-C₆)-alkyl, wherein alkyl is optionally substituted one or more times by hydroxyl, (C₁-C₄)-alkoxy, F, Cl or Br and wherein aryl is optionally substituted 1-3 times by hydroxyl, (C₁-C₄)-alkoxy, (C₁-C₄)-alkyl, F, Cl, Br, NO₂, -NR⁹R¹⁰, -C(O)OH, -C(O)O-(C₁-C₆)-alkyl or -C(O)NR⁹R¹⁰;

R⁹ and R¹⁰ independently of one another are hydrogen, (C₁-C₁₈)-alkyl, (C₁-C₁₈)-alkenyl, (C₁-C₁₈)-alkynyl, (C₆-C₁₂)-aryl, (C₆-C₁₂)-aryl-(C₁-C₆)-alkyl, where alkyl is optionally substituted one or more times by hydroxyl, (C₁-C₄)-alkoxy, F, Cl or Br; or R⁹ and R¹⁰ form a 4 to 7-membered ring together with the N atom in -NR⁹R¹⁰;

Q and Q' independently of one another are hydrogen, R⁸, modified or unmodified oligonucleotides or conjugates which a) favorably affect the properties of antisense oligonucleotides, b) affect the properties of triple helix-forming oligonucleotides c) serve as a label of a DNA probe, or d) during the hybridization of the oligonucleotide analog to the target nucleic acid, attack the target nucleic acid with binding or cross-linking; or Q and Q' alone or together are a single bond in a cyclic molecule; or Q and Q', when neither is

hydrogen, can be linked together to form a cyclic molecule.

- 5 2. A compound of formula I as claimed in claim 1, in which
- n is a number from zero to 50;
- B independently of one another is a natural nucleobase or
- 10 an unnatural nucleobase;
- L is N;
- A is a group of the formula IIb, in which
- 15 r = 1 and s is zero, and R², R³ = H and Y' = O and M is
- a single bond;
- D and G each independently represent CHR⁵;
- 20 R⁵ is hydrogen;
- X is -O-;
- Y is =O;
- 25 Z is hydroxyl, methoxy, ethoxy, (4-nitrophenyl)ethoxy,
- propoxy, isopropoxy, butoxy, pentoxy, phenoxy or
- allyloxy;
- 30 Q and Q' independently of one another are hydrogen, R²,
- or modified or unmodified oligonucleotides, wherein
- said modified oligonucleotides are selected from
- a) oligonucleotides wherein 3'- and/or 5'-phosphoric
- 35 acid diester bridges are completely or partially replaced by
- any of phosphorothioate, phosphorodithioate, NR'R'-

phosphoramidate, phosphate O-methyl ester, phosphate O-ethyl ester, phosphate O-isopropyl ester, methylphosphonate and phenylphosphonate bridges;

5 b) oligonucleotides wherein one, two or three 3'- or 5'-phosphoric acid diester bridges in the pyrimidine positions and either at the 5' end, at the 3' end, or the 5' end and 3' end are replaced by any of formacetals and 3'-thioformacetals;

10 c) oligonucleotides wherein the sugar phosphate backbone is completely or partially replaced by any of "PNAs" and PNA-DNA hybrids;

15 d) oligonucleotides wherein β -D-2'-deoxyribose units are completely or partially replaced by any of 2'-F-2'-deoxyribose, 2'-O-(C₁-C₆)-alkylribose, 2'-O-(C₂-C₆)-alkenylribose and 2'-NH₂-2'-deoxyribose; and

20 e) oligonucleotides wherein natural nucleoside bases are completely or partially replaced by any of 5-(C₁-C₆)-alkyluracil, 5-(C₂-C₆)-alkenyluracil, 5-(C₂-C₆)-alkynyluracil, 5-(C₁-C₆)-alkylcytosine, 5-(C₂-C₆)-alkenylcytosine, 5-(C₂-C₆)-alkynylcytosine, 5-fluorouracil, 5-fluorocytosine, 25 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine, 7-deaza-7-(C₂-C₁)-alkynylguanine, 7-deaza-7-(C₂-C₁)-alkenylguanine, 7-deaza-7-(C₂-C₁)-alkynyladenine, 7-deaza-7-(C₂-C₁)-alkenyladenine, 7-deaza-7-(C₁-C₁)-alkylguanine, 7-deaza-7-(C₁-C₁)-alkyladenine, 7-deaza-7-bromoguanine, and 7-deaza-7-bromoadenine. 30

3. A compound of formula I as claimed in claim 2, in which

n is a number from 0 to 30;

35 Q and Q' independently of one another are hydrogen,

modified or unmodified oligonucleotides or R^0 , in which R^0 is H, (C_1-C_6) -alkyl, phenyl or 2-(4-nitrophenyl)-ethyl, wherein said modified oligonucleotides are selected from

5

a) oligonucleotides wherein 3'- and/or 5'-phosphoric acid diester bridges are completely or partially replaced by any of phosphorothioate, phosphorodithioate and methylphosphonate bridges;

10

b) oligonucleotides wherein one, two or three 3'- or 5'-phosphoric acid diester bridges are replaced at the 5'- and at the 3'-end;

15

c) oligonucleotides wherein the sugar phosphate backbone is completely or partially replaced by "PNAs" and PNA-DNA hybrids;

20

d) oligonucleotides wherein β -D-2'-deoxyribose units are completely or partially replaced by any of 2'-F-2'-deoxyribose, 2'-O- (C_1-C_4) alkylribose, 2'-O- (C_2-C_4) alkenylribose and 2'-NH₂-2'-deoxyribose; and

25

e) oligonucleotides wherein natural nucleoside bases are completely or partially replaced by any of 5- (C_3-C_6) -alkyluracil, 5- (C_2-C_6) -alkenyluracil, 5- (C_2-C_6) -alkynyluracil, 5- (C_1-C_6) -alkylcytosine, 5- (C_2-C_6) -alkenylcytosine, 5- (C_2-C_6) -alkynylcytosine, 7-deaza-7- (C_2-C_1) -alkynylguanine, 7-deaza-7- (C_2-C_1) -alkynyladenine, 7-deaza-7- (C_2-C_1) -alkenylguanine, 7-deaza-7- (C_2-C_1) -alkenyladenine, 7-deaza-7- (C_1-C_1) -alkylguanine, 7-deaza-7- (C_1-C_1) -alkyladenine, 7-deaza-7-bromoguanine, and 7-deaza-7-bromoadenine.

30

35

4. A compound of formula I as claimed in claim 3, wherein

n is a number from 0 to 25;

B independently of one another is a natural nucleobase;

5 Z is hydroxyl, ethoxy, (4-nitrophenyl)ethoxy or phenoxy;

Q and Q' independently of one another are hydrogen,
modified or unmodified oligonucleotides, or R⁸, in
which R⁸ is H, (C₁-C₆)-alkyl, phenyl or 2-(4-nitro-
10 phenyl)ethyl, wherein said modified oligonucleotides
are selected from:

a) oligonucleotides wherein 3'- and/or 5'-phosphoric
acid diester bridges are completely or partially replaced by
15 phosphorothioate bridges;

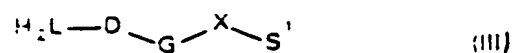
c) oligonucleotides wherein the sugar phosphate
backbone is completely or partially replaced by any of
"PNAs" and PNA-DNA hybrids;

20 d) oligonucleotides wherein β -D-2'-deoxyribose units
are completely or partially replaced by any of 2'-O-methyl-,
2'-O-allyl- and 2'-O-butylribose;

25 e) oligonucleotides wherein natural nucleoside bases
are completely or partially replaced by any of 5-
hexynylcytosine, 5-hexynyluracil, 7-deaza-7-propynylguanine,
7-deaza-7-propynyladenine, 7-deaza-7-methylguanine, 7-deaza-
7-methyladenine, 7-deaza-7-bromoguanine, and 7-deaza-7-
30 bromoadenine.

5. A process for the preparation of a compound of
formula I in claim 1, which comprises:

a₁) reacting a compound of formula III

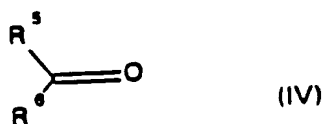


in which

D, G, L and X have the same meanings as in claim 1 and

S' is a protective group,

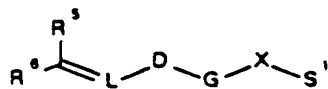
with a compound of formula IV



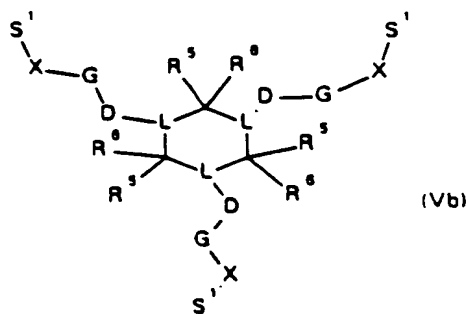
in which

R⁵ and R⁶ have the same meanings as in claim 1,

in an organic solvent, at a temperature sufficient to form a compound of formula Va or Vb

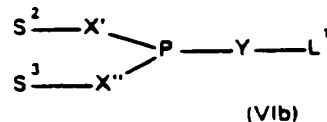
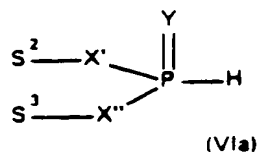


(Va)



(Vb)

b₁) reacting a compound of the formula Va or Vb with a compound of the formula VIa or VIb



in which

Y has the same meaning as in claim 1,

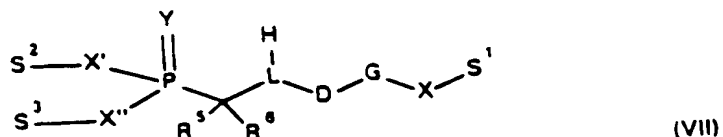
X' and X'' independently of one another are defined as X as in claim 1,

S² and S³ independently of one another are protective groups,

and

L¹ is a leaving group,

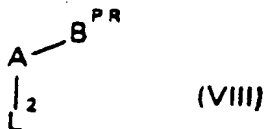
in an organic solvent, optionally with addition of a base, at a temperature sufficient to form a compound of formula VII



in which

D, G, L, R⁵, R⁶, X, and Y have the same meaning as in claim 1;

c₁) reacting a compound of formula VII with a compound of formula VIII



in which

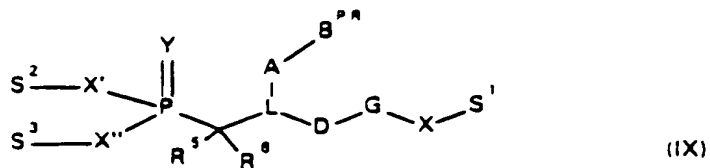
A has the same meaning as claim 1,

B^{PR} has the same meaning as B in claim 1, optionally present in protected form, and

L² is a leaving group, or, if A is formula IIb, L² is a leaving group or OH;

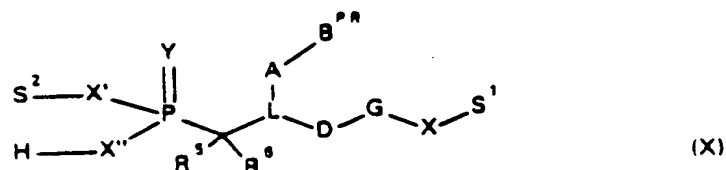
in an organic solvent, optionally with addition of base, or without addition of base and optionally with addition of a coupling reagent for the coupling of peptide bonds,

at a temperature sufficient to form to give a compound of formula IX



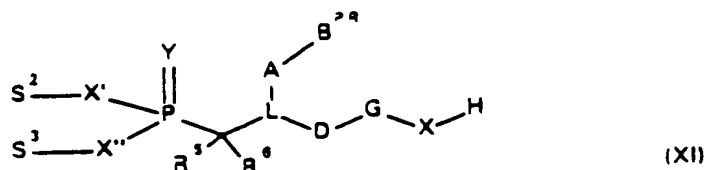
d₁) removing the protective group S¹ from the compound of formula IX

to form a compound of formula X



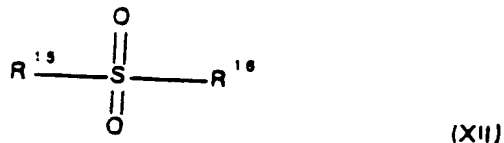
e₁) removing the protective group S¹ from another compound of formula IX

to form a compound of formula XI



said steps d₁) and e₁) being carried out in any order

f₁) reacting the compound of formula XI with the compound of formula X in an organic solvent, with addition of a coupling reagent or a compound of formula XII



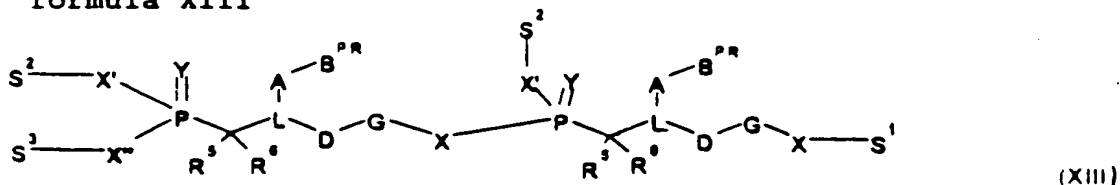
in which

R¹⁵ is (C6-C12)-aryl, optionally substituted one to four times by (C1-C6)-alkyl, (C1-C6)-alkoxy, nitro, chlorine or bromine and where one to 3 carbon atoms are optionally substituted by heteroatoms, and

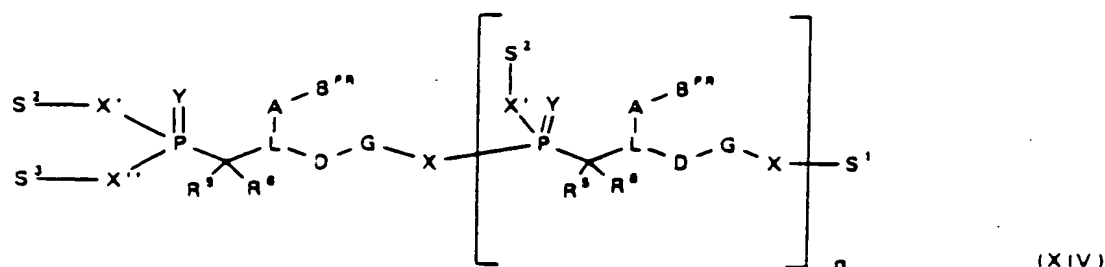
R¹⁶ is a leaving group,

optionally with addition of a catalyst,

and at a temperature sufficient to form a compound of formula XIII



g₁) starting from the compound of formula XIII, repeating steps e₁) and f₁) as necessary, to form a compound of formula XIV



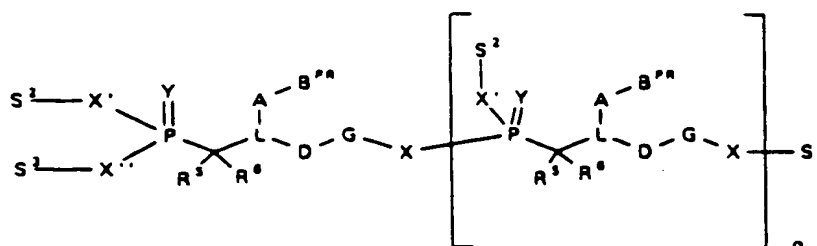
wherein $n = 1$ to 100 ,

h₁) in any order, removing the protective group S¹ and optionally introducing the group Q'; removing the protective group on B^{PR}; removing the protective groups S² and optionally introducing Q'', R⁶, R³, and R¹⁰; removing the protective group S³ and optionally introducing the group Q,

i₁) optionally cyclizing, to form the compound of formula I.

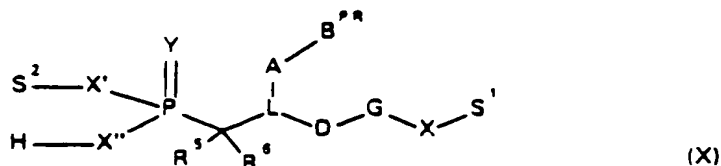
6. A process for the preparation of a compound of formula I as claimed in claim 1, in which n is 1 to 100, which comprises,

a₂) removing the protective group S¹ from a compound of formula XV,



7. A process for the preparation of a compound of formula I as claimed in claim 1, which comprises

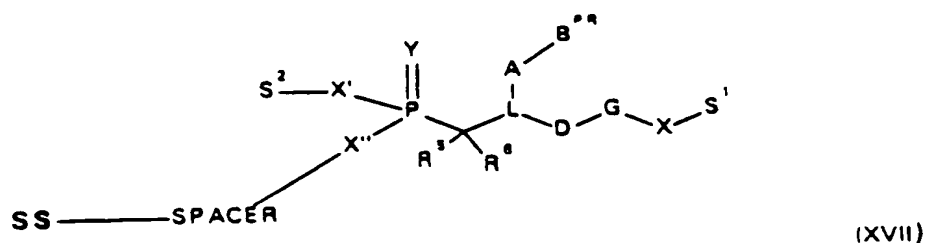
a.) coupling a compound of formula X



in which

A, D, G, L, R⁵, R⁶, X, and Y are as defined in claim 1; B^{PR} has the same meaning as B in claim 1, optionally present in protected form, S¹ and S², independently of one another, are protective groups; and, X' and X'' independently of one another are defined as X in claim 1;

to a solid support (SS) via a SPACER to form a compound of formula XVII,



in which

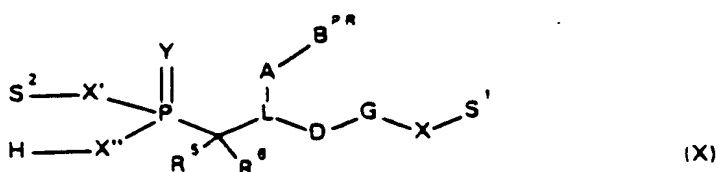
SS is a solid support suitable for solid-phase synthesis, and

SPACER is a group removable from the support after synthesis

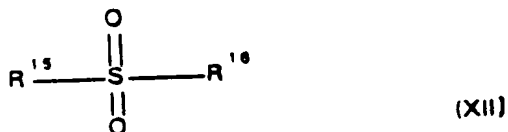
has taken place, or SPACER is a bifunctional conjugate molecule Q linked to the solid support via removable groups,

b₃) removing the protective group S¹ from the compound of formula XVII

c₃) reacting the resulting compound with a compound of formula X



in an organic solvent, with addition of a coupling reagent in a compound of formula XII

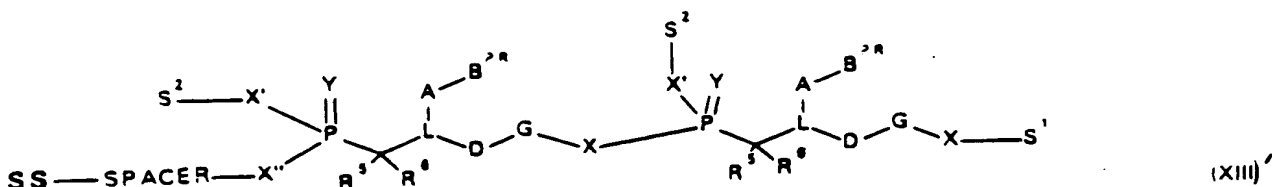


in which

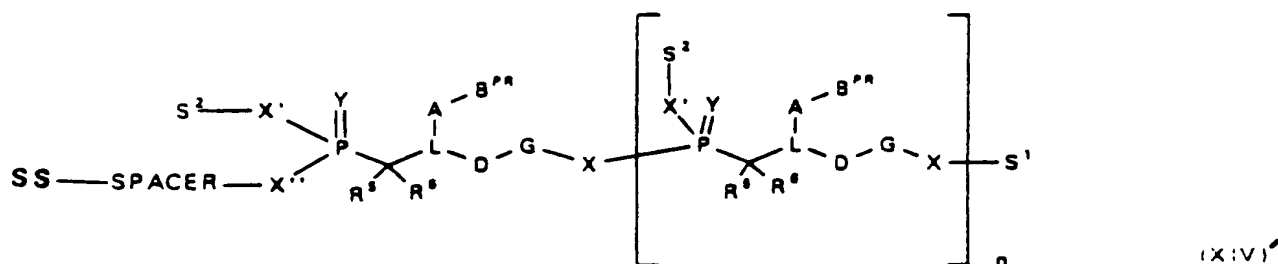
R¹⁵ is (C₆-C₁₂)-aryl, optionally substituted one to four times by (C₁-C₆)-alkyl, (C₁-C₆)-alkoxy, nitro, chlorine or bromine and where one to 3 carbon atoms are optionally substituted by heteroatoms, and

R^{16} is a leaving group,

optionally with addition of a catalyst, to form a compound of formula XIII'



15 d₃) starting from the compound (XIII') resulting from said step c₃), repeating steps b₃) and c₃) is necessary to form a compound of formula XIV';



25 e₃) in any order, removing the protective groups on B^{PR} ; removing the protective group S^1 and optionally coupling conjugate Q' ; removing the resulting compound of step d₃ from the solid support and optionally coupling conjugate Q ; removing the protective groups S^2 and optionally introducing Q^{11} , R^8 , R^9 and R^{10} ,

30 f₃) optionally cyclizing, to form a compound of formula I of claim 1.

35 8. A method to inhibit gene expression comprising the step of administering to a host a therapeutically active

composition comprising a compound of formula I of claim 1 and a physiologically acceptable excipient.

5 9. A method for diagnosing the presence of a nucleic acid molecule in a biological sample comprising adding a compound of formula I of claim 1 to said biological sample.

10 10. The compound of claim 1, wherein R¹ is hydrogen or methyl.

11. The compound of claim 1, wherein R² and R³ are both hydrogen.

15 12. The compound of claim 1, wherein R⁰ is hydrogen, (C₁-C₆)-alkyl, (C₆-C₁₂)-aryl, or (C₆-C₁₂)-aryl-(C₁-C₆)-alkyl, wherein the aryl is optionally monosubstituted by (C₁-C₄)-alkoxy, (C₁-C₄)-alkyl, F, Cl, Br, or NO₂.

20 13. The compound of claim 1, wherein R⁰ is hydrogen, (C₁-C₆)-alkyl, phenyl, or 2-(4-nitrophenyl)ethyl.

25 14. The process of claim 5, wherein S¹ is a protective group selected from dimethoxy~~dimethoxy~~trityl, monomethoxytrityl, trityl, pixyl, tert-butoxycarbonyl, or fluorenylmethoxy-carbonyl.

30 15. The process of claim 5, wherein said temperature sufficient to form a compound of formula Va or Vb is 0°C to 100°C.

35 16. The process of claim 5, wherein said temperature sufficient to form a compound of formula VII is from 0°C to 100°C.

17. The process of claim 5, wherein said temperature sufficient to form a compound of formula IX is from -20°C to 100°C.

5 18. The process of claim 5, wherein said temperature sufficient to form a compound of formula XII is from -20°C to 100°C.

10 19. The process of claim 5, wherein L¹ is a (C₁-C₄)-alkyl.

20. The process of claim 5, wherein the coupling reagents can be prepared *in situ*.

15 21. The process of claim 5, wherein the coupling reagent is prepared separately in a solvent and then added in said step (f₁).

20 22. A method for the treatment of viruses comprising the step of administering to a patient a composition comprising a compound of formula I of claim 1 with a physiologically acceptable excipient.

25 23. A method of treating cancer or restenosis comprising the step of administering to a patient an effective amount of a composition comprising a compound of formula I of claim 1 and a physiologically acceptable excipient.

30 24. The method of claim 22, wherein said virus is HIV, HSV-1, HSV-2, influenza, VSV, hepatitis B, or papilloma virus.

35 25. A method of treating medical conditions resulting from integrins or cell-cell adhesion receptors comprising administering to a patient an effective amount of a

composition comprising a compound of formula I of claim 1 and a physiologically acceptable excipient.

5 26. A method for the treatment of medical conditions induced by growth factors comprising the step of administering to a patient an effective amount of a composition comprising a compound of formula I of claim 1 and a physiologically acceptable excipient.

10 27. The method of claim 26, wherein said growth factor is TNF- α .

15 28. The process of claim 5, wherein said base is a complex base or an uncharged peralkylated polyaminophosphazene base.

29. The compound of claim 1, wherein Q is an unmodified or modified oligonucleotide and Q' is hydrogen.

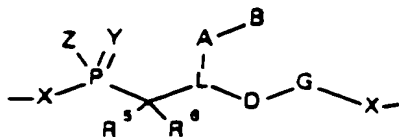
20 30. The compound of claim 1, wherein Q is hydrogen and Q' is an unmodified or modified oligonucleotide.

25 31. The compound of claim 1, wherein Q and Q', independently of one another, are an unmodified or modified oligonucleotide.

32. A compound having one of the formulae:

30 5'- Q¹¹-OLIGO-(PMENA-OLIGO)a-Q¹²;
5'- Q¹³-PMENA-OLIGO-PMENA-Q¹⁴; or
5'- Q¹⁵-PMENA-(OLIGO-PMENA)a-Q¹⁶; or
5'- OLIGO-(PMENA-OLIGO)a;

35 wherein a is 1-20, OLIGO represents an unmodified or modified oligonucleotide, and PMENA represents a compound of formula I':



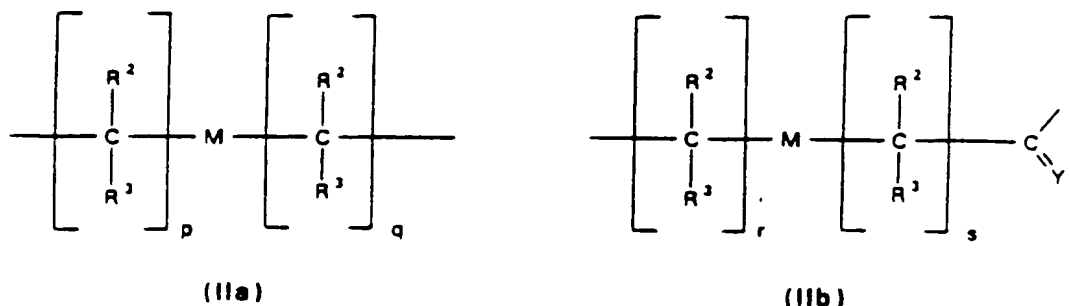
(II)'

5

in which

- 10 B independently of one another is hydrogen, hydroxyl, (C₁-C₂₀)-alkyl, (C₁-C₂₀)-alkoxy, (C₁-C₂₀)-alkylthio, (C₆-C₂₀)-aryl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkyl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkoxy, (C₆-C₂₀)-aryl-(C₁-C₆)-alkylthio, an aromatic group or a heterocyclic group, wherein alkyl, aryl, aromatic or heterocyclic group is optionally
- 15 substituted one or more times by hydroxyl, (C₁-C₄)-alkoxy, -NR⁹R¹⁰, -C(O)OH, oxo, -C(O)OR⁹, -C(O)NR⁹R¹⁰, -CN, -F, -Cl, -Br, -NO₂, (C₂-C₆)-alkoxyalkyl, -S(O)_nR⁹, -(C₁-C₆)-alkyl-S(O)_nR⁹, -NHC(=NH)NHR⁹, -C(=NH)NHR⁹, -NR⁹C(=O)R⁹, =NOR⁹, NR⁹C(=O)OR¹⁰, -OC(=O)NR⁹R¹⁰ or
- 20 -NR⁹C(=O)NR⁹R¹⁰,
- B independently of one another is a natural nucleobase, an unnatural nucleobase or a reporter ligand;
- 25 A-B is a D- or L-amino acid condensed on via the carboxyl group or peptides consisting of amino acids having a length of up to 5 amino acid residues,
- L independently of one another is N or R¹N⁺, and
- 30 R¹ is hydrogen or (C₁-C₆)-alkyl optionally substituted by hydroxyl, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio or amino;

A independently of one another is a single bond, a methylene group or a group of formula IIa or IIb;



Y' is =O, =S, =CH₂, =C(CH₃)₂ or =NR¹, where R¹ is as defined above;

M is a single bond, -O-, -S- or -NR¹-, where R¹ is as defined above;

R² and R³ independently of one another are hydrogen, hydroxyl, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio, amino, halogen, or (C₁-C₆)-alkyl optionally substituted by hydroxyl, (C₁-C₆)-alkoxy or (C₁-C₆)-alkylthio;

p and q independently of one another are zero to 5;

r and s independently of one another are zero to 5;

D and G each independently represent CR⁵R⁶ which can be the same or different;

R⁵ and R⁶ independently of one another are hydrogen, (C₁-C₆)-alkyl, (C₆-C₂₀)-aryl, (C₆-C₂₀)-aryl-(C₁-C₆)-alkyl, hydroxyl, (C₁-C₆)-alkoxy, (C₁-C₆)-alkylthio, wherein alkyl and aryl are optionally substituted by SR¹ or NR¹R^{1'}, where R¹ is as defined above and R^{1'} independently of R¹ has the same meaning as R¹;

X is -O-, -S- or -NR¹-, in which R¹ is as defined above;

Y is =O or =S;

Z is $-OR^8$, $-NR^9R^{10}$ or $X'Q''$, where X' is defined as X and Q'' is hydrogen, R^8 , modified or unmodified oligonucleotides or conjugates which a) favorably affect the properties of antisense oligonucleotides, b) affect the properties of triple helix-forming oligonucleotides c) serve as a label of a DNA probe, or d) during the hybridization of the oligonucleotide analog to the target nucleic acid, attack the target nucleic acid with binding or crosslinking;

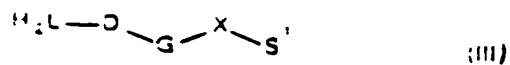
R^8 is hydrogen, (C_1-C_{18}) -alkyl, (C_2-C_{18}) -alkenyl, (C_3-C_{18}) -alkynyl, (C_6-C_{12}) -aryl, (C_6-C_{12}) -aryl- (C_1-C_6) -alkyl, wherein alkyl is optionally substituted one or more times by hydroxyl, (C_1-C_4) -alkoxy, F, Cl or Br and wherein aryl is optionally substituted 1-3 times by hydroxyl, (C_1-C_4) -alkoxy, (C_1-C_4) -alkyl, F, Cl, Br, NO_2 , $-NR^9R^{10}$, $-C(O)OH$, $-C(O)O-(C_1-C_6)$ -alkyl or $-C(O)NR^9R^{10}$;

R^9 and R^{10} independently of one another are hydrogen, (C_1-C_{18}) -alkyl, (C_1-C_{18}) -alkenyl, (C_1-C_{18}) -alkynyl, (C_6-C_{12}) -aryl, (C_6-C_{12}) -aryl- (C_1-C_6) -alkyl, where alkyl is optionally substituted one or more times by hydroxyl, (C_1-C_4) -alkoxy, F, Cl or Br; or R^9 and R^{10} form a 4 to 7-membered ring together with the N atom in $-NR^9R^{10}$;

and wherein Q^{13} and Q^{14} either both represent hydrogen or together form a bond in a cyclic molecule; Q^{15} and Q^{16} either both represent hydrogen or together form a bond in a cyclic molecule; and Q^{11} and Q^{12} form a single bond in a cyclic molecule.

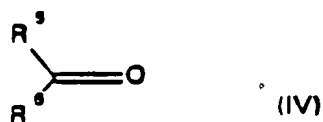
33. A process for the preparation of a compound of formula I in claim 1, which comprises:

a₁) reacting a compound of formula III



in which

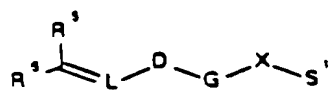
D, G, L and X have the same meanings as in claim 1 and
S¹ is a protective group,
with a compound of formula IV



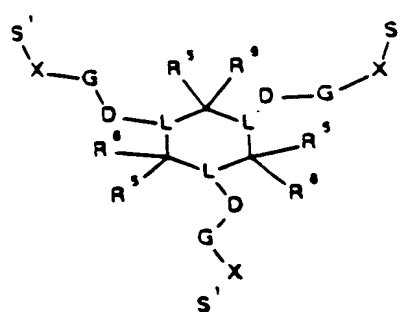
in which

R⁵ and R⁶ have the same meanings as in claim 1,

in an organic solvent, at a temperature sufficient to form a
compound of formula Va or Vb

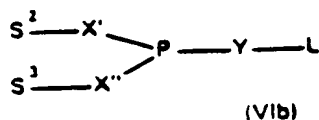
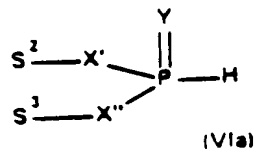


(Va)



Vb)

b₁) reacting a compound of the formula Va or Vb with a compound of the formula VIa or VIb



in which

Y has the same meaning as in claim 1,

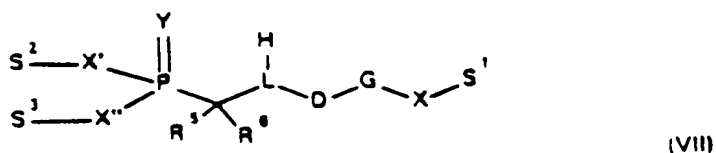
X' and X'' independently of one another are defined as X as in claim 1,

S² and S³ independently of one another are protective groups,

and

L¹ is a leaving group,

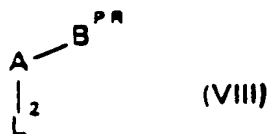
in an organic solvent, optionally with addition of a base, at a temperature sufficient to form a compound of formula VII



in which

D, G, L, R⁵, R⁶, X, and Y have the same meaning as in claim 1;

5 c₁) reacting a compound of formula VII with a compound of formula VIII



in which

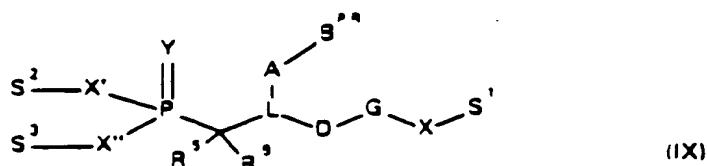
15 A has the same meaning as claim 1,

B^{PR} has the same meaning as B in claim 1, optionally present in protected form, and

L² is a leaving group, or, if A is formula IIb, L² is a leaving group or OH;

20 in an organic solvent, optionally with addition of base, or without addition of base and optionally with addition of a coupling reagent for the coupling of peptide bonds,

at a temperature sufficient to form to give a compound of formula IX

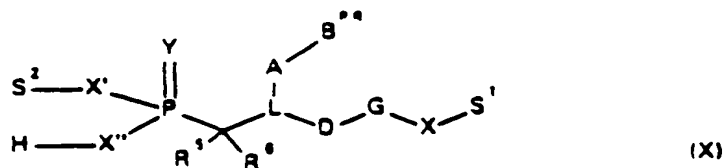


d₁) in any order, removing the protective group S¹ and optionally introducing group Q'; removing protective group S² and optionally introducing Q'', R⁸, R⁹ and R¹⁰; removing the protective groups on B^{PR}; removing the protective group S³ from the compound of formula IX, and optionally introducing the group Q,

e₁) optionally cyclizing, to form the compound of formula I.

34. A process for the preparation of a compound of formula I as claimed in claim 1, which comprises

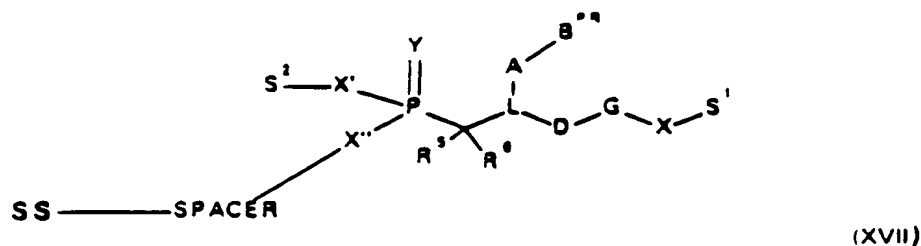
a₁) coupling a compound of formula X



in which

A, D, G, L, R⁵, R⁶, X, and Y are as defined in claim 1; B^{PR} has the same meaning as B in claim 1, optionally present in protected form, S¹ and S², independently of one another, are protective groups; and, X' and X'' independently of one another are defined as X in claim 1;

to a solid support (SS) via a SPACER to form a compound of formula XVII,



in which

SS is a solid support suitable for solid-phase synthesis, and

SPACER is a group removable from the support after synthesis has taken place, or SPACER is a bifunctional conjugate molecule Q linked to the solid support via removable groups,

b,) in any order, removing the protective group on B^{PR}; removing the protective group S¹ and optionally introducing the group Q'; removing the protective group S² and optionally introducing Q'', R⁵, R⁶ and R¹⁰; removing the resulting compound of step a,) from the solid support and optionally introducing the group Q,

c,) optionally cyclizing, to form the compound of formula I.

35. A pharmaceutical composition comprising a compound of the formula I as claimed in any one of claims 1 and 10 to 13 and a pharmaceutically acceptable excipient.

5 36. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 to inhibit gene expression.

10 37. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 to treat viruses.

15 38. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 to treat HIV, HSV-1, HSV-2, influenza, VSV, hepatitis B, or papilloma virus.

 39. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 to treat cancer or restenosis.

20 40. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 to treat medical conditions resulting from integrins or cell-cell adhesion receptors.

25 41. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 to treat medical conditions induced by growth factors.

30 42. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 to treat medical conditions induced by TNF- α .

35 43. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 in the preparation of a pharmaceutical composition for inhibiting gene expression.

 44. Use of a compound of the formula I as claimed in

any one of claims 1 and 10 to 13 in the preparation of a pharmaceutical composition for treating viruses.

5 45. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 in the preparation of a pharmaceutical composition for treating HIV, HSV-1, HSV-2, influenza, VSV, hepatitis B, or papilloma virus.

10 46. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 in the preparation of a pharmaceutical composition for treating cancer or restenosis.

15 47. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 in the preparation of a pharmaceutical composition for treating medical conditions resulting from integrins or cell-cell adhesion receptors.

20 48. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 in the preparation of a pharmaceutical composition for treating medical conditions induced by growth factors.

25 49. Use of a compound of the formula I as claimed in any one of claims 1 and 10 to 13 in the preparation of a pharmaceutical composition for treating medical conditions induced by TNF- α .

Fig. 1 UV-Absorbance profile versus temperature for [PMENA-t9]
in the presence of 1 eq. (dA)g

10 mM HEPES, 20 mM MgCl₂; 1M NaCl; pH 7,5

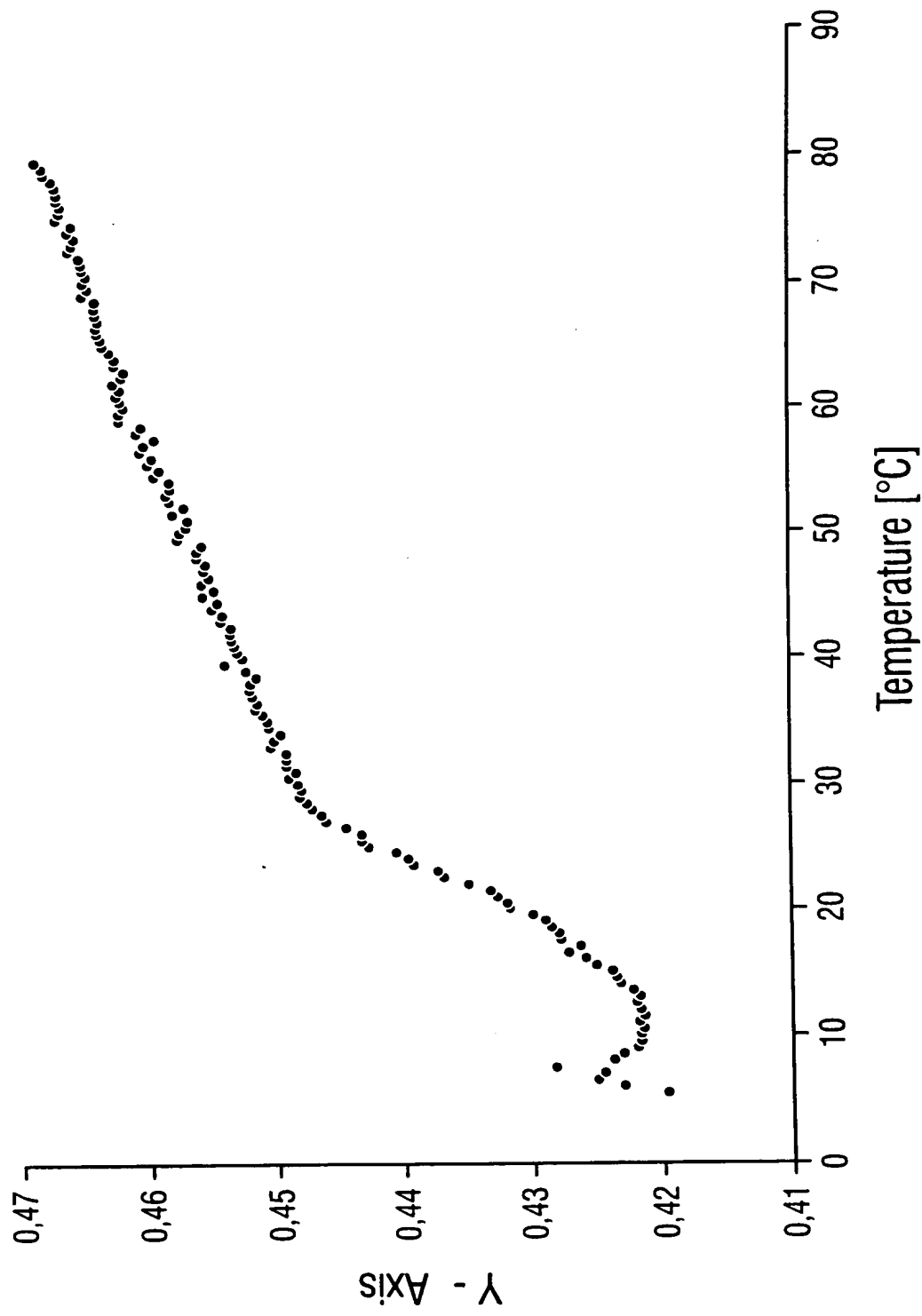


Fig. 2

Proof of PMENA-DNA Binding by Gel-Shift

